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Serotonergic modulation of odour-evoked neural activity in the olfactory bulb of the sea lamprey (Petromyzon marinus)

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Serotonergic modulation of odour-evoked neural activity in the olfactory bulb of the sea lamprey (*Petromyzon marinus)*

By

Karl C. Boyes

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Abstract

Serotonergic (5-hydroxytryptamine, 5-HT) fibers are distributed throughout the olfactory nerve and olfactory bulb of the sea lamprey. This study tested for 5-HT modulation of olfactory sensory responses to pheromone, amino acid and bile acid odours in the dorsal and lateral regions of the olfactory bulb by recording local field potentials. The peak amplitude of dorsal and lateral olfactory bulb responses to amino acid odours declined during 5-HT bath-application. The peak amplitude of responses to all odours increased during pharmacological blockade of the 5-HT1a receptor, but was not affected when 5-HT was added to the 5-HT1a antagonist, spiperone. The 5-HT1a antagonist, s(-)-uh-301, lengthened responses and had a larger number of peaks to all odours and spiperone lengthened responses and had a larger number of peaks to amino acids. We conclude that 5-HT attenuates odour responses in olfactory bulb via the 5HT1a receptor and suggest that 5-HT may also modulate olfactory-mediated behaviours.

Dedicated to my parents, Paul and Carol Boyes, and brother, Erik Boyes.
Thank-you for your constant love and support.

Acknowledgements

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List of Abbreviations

3kACA	3-keto allocholic acid
3kPZS	3-keto petromyzonol sulphate
5-HT	5-hydroxytryptamine
AA	Amino acid
AC	Adenylyl cyclase
ACA	Allocholic acid
ATP	Adenosine triphosphate
cAMP	Cyclic adenosine monophosphate
CNG	Cyclic nucleotide gated
DRN	Dorsal raphe nucleus
GI	Gastrointestinal
GIRK	G-protein inward rectifying potassium
GPCR	G-protein coupled receptor
LFP	Local field potential
LP	Lamina propria
MOE	Main olfactory epithelium
OB	Olfactory bulb
OE	Olfactory epithelium
ON	Olfactory nerve
OSN	Olfactory sensory neuron
PADS	Petromyzonamine disulphate
PSDS	Petromyzonolsterol disulphate
PZS	Petromyzonol sulphate
S(-)-UH-301	S(-)-5-fluoro-8-hydroxy-dipropylaminotetralin hydrochloride
TCA	Taurocholic Acid

Chapter 1

Introduction

1.1 General Introduction

The environment that surrounds an organism is the source of a variety of different types of sensory information. Evolution has developed a set of sophisticated sensory systems that decode this information and direct it to the appropriate brain regions (Macaluso and Driver, 2005), which produce the behavioural responses that are critical for reproduction and survival. These include: the somatosensory, visual, auditory and olfactory systems. Significant advancements have been made in determining how odour information leads to a behavioural response in fish, insects and rodents. The olfactory-locomotor neural substrate has been demonstrated in an invertebrate (Gray et al., 2005) and the sea lamprey (Derjean et al., 2010). However, olfactory and other sensory signals are subject to neuromodulation by norepinephrine, acetylcholine, dopamine, 5-HT, GABA and a wide range of peptides (Hasselmo 1994) that enable neural activity to be enhanced or suppressed. These neuromodulators have the ability to excite or inhibit the transmission of signals being transported along the olfactory-motor pathway. 5-HT (5-hydroxytryptamine) is a neuromodulator that has been shown to be distributed throughout the olfactory bulb (OB) and has modulatory effects on olfactory-mediated neural processes and behaviours in many vertebrate species such as the rat (McLean et al., 1993 Langdon et al., 1997; Hardy et al., 2005), the bat (Ganesh et al., 2010), the rabbit (Baumgarten et al., 1963; Bloom et al., 1964) and the mouse (Petzold et al., 2009). 5-HT fibers have also been discovered to be distributed throughout the lamprey olfactory bulb (Adrio et al., 1999; Zielinski et al., 2000; Frontini et al., 2003; Barreiro Iglesias et al., 2009; Barreiro-Iglesias et al., 2012). While the inhibitory effects caused by 5-HT modulation are well documented on locomotor activity in the lamprey (Grillner et al., 1985; Harris-Warrick et al., 1985; Christenson et al., 1989; Wallen et al., 1989; Buchanan and Grillner 1991; Zhang et al., 1996; El Manira et al., 1997;

Grillner et al., 2003; Schwartz et al., 2005), nothing is known about the neural effect 5-HT has on olfactory modulation in the OB. Since the sea lamprey is one of the oldest extant vertebrates and it has 5-HT distributed throughout its primary olfactory pathway for its entire lifecycle (Zielinski et al., 2000; Frontini et al., 2003), it is used as model species for this thesis to study the effects of 5-HT modulation on olfactory responses in the OB. The objective of this chapter is to review the olfactory bulb and modulation, describe previous studies that investigated 5-HT modulation of olfaction, examine 5-HT modulation in different systems of the lamprey and discuss the logic and predictions behind the 5-HT modulatory experiments conducted in this study.

1.2 Olfaction

The olfactory system of vertebrates is capable of discriminating a wide variety of structurally diverse odours. There has been an evolutionary convergence among signalling pathways in olfactory systems towards a conserved organization (Hildebrand et al., 1997). Odour processing in vertebrates occurs at distinct anatomical structures as the olfactory information progresses. Olfactory sensory neurons (OSN's) located in the main olfactory epithelium (MOE) detect odour molecules, send the information to the OB in the brain, which sends the information to higher brain structures (Buck 1996). Ciliated and microvillus OSN's are present in many vertebrates, although a third known as crypt cells has been detected in others (Hansen and Finger 2000). The olfactory system of mammals has two subdivisions known as the main olfactory system, located in the main olfactory epithelium, and the accessory olfactory organ, located in the vomeronasal organ. The main olfactory system contains ciliated OSN's, while the VNO contain microvillous OSN's (Ma 2007). Teleost fish have ciliated and microvillous OSN's in the peripheral olfactory organ (Zielinski and Hara 2006), while lampreys only have ciliated OSN's

in the olfactory epithelium (OE)(Vandenbossche et al., 1995). The OSN's send their axons into glomeruli, conglomerates of neuropil, in the OB, that also contain the dendrites of mitral cells, which are the main projection cell in the OB (Firestein 2001) (Figure 1.1). In mammals, all the OSN's that express a particular odour receptor converge at one or a few glomeruli in the OB (Mombaerts 1996). In the glomeruli, the OSN's release the excitatory neurotransmitter glutamate from their axon terminals onto the dendrites of mitral cells. Within the lamprey OB, there are 4 different layers (Figure 1.2). The first is the layer of the olfactory fibers, which contain the OSN's that will terminate in the olfactory glomeruli. The second layer is the glomerular layer, which contains the glomeruli, OSN's and mitral cells (Iwahori et al., 1987). The third layer is the granule cell layer, which contain granule cells that release the inhibitory neurotransmitter GABA and have projections into the glomerular layer of the OB. The fourth layer is the ependymal layer, which contain ependymal cells that also have projections into the glomerular layer (Iwahori et al., 1987).

1.3 5-HT modulation

Neurotransmission is the process by which one neuron, the presynaptic neuron, communicates with another neuron, the postsynaptic neuron. The presynaptic neuron conveys information to the postsynaptic neuron when the firing of an action potential causes neurotransmitter molecules to be released by the presynaptic neuron, which bind to and activate receptors on the postsynaptic neuron. In contrast, neuromodulation occurs when an interneuron uses modulatory neurotransmitters, such as dopamine or GABA, to regulate presynaptic or postsynaptic neurons partaking in neurotransmission. The result is either an enhancement or

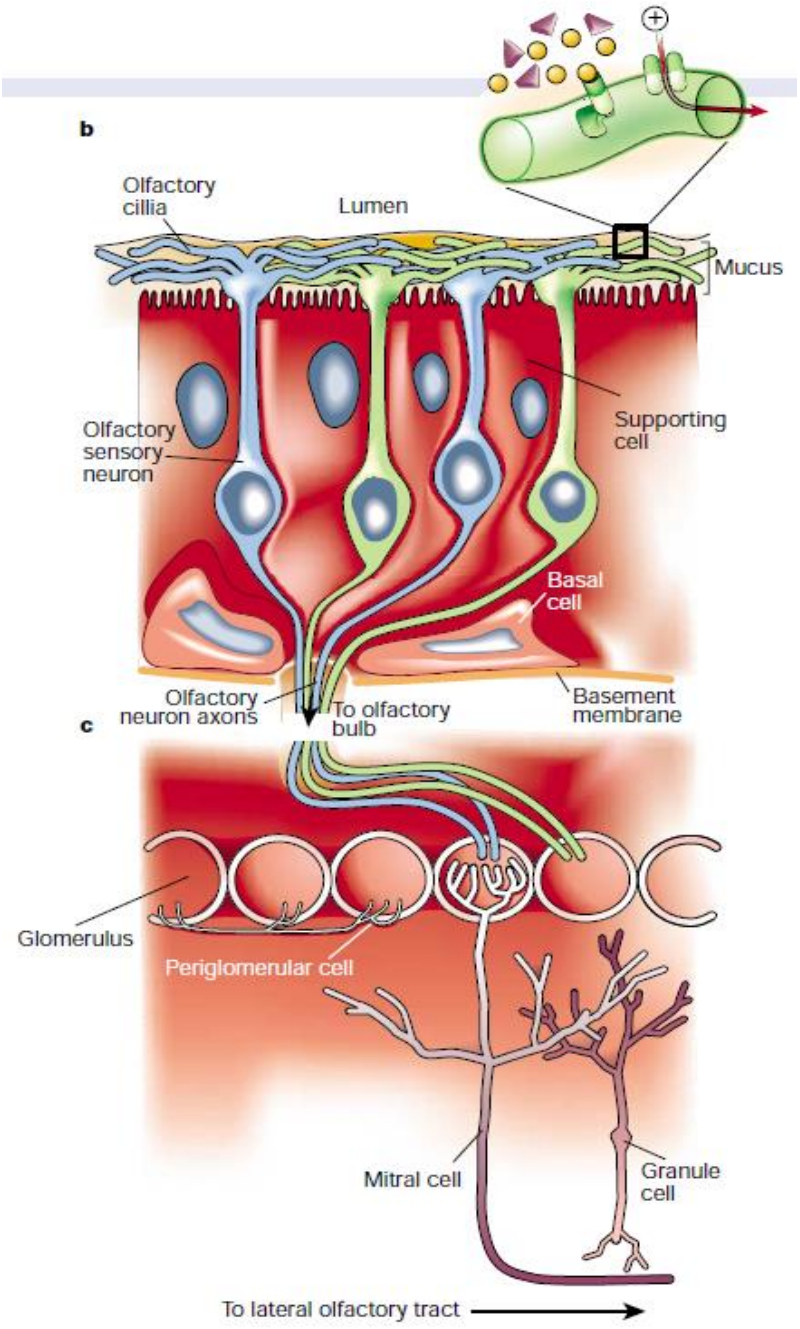


Figure 1.1. The progression of odour information by the OSN's in the olfactory epithelium to the olfactory bulb (OB). Olfactory sensory neurons (blue and green) bind odour molecules to their receptors in the olfactory epithelium, transmit this information along their axons and synapse in glomeruli in the OB. Mitral cells (white), also in the glomeruli, retrieve this information and deliver it to higher brain structures. Periglomerular cells and granule cells are interneurons that allow for lateral processing of the information. Illustration from Firestein, 2001. Nature Vol 413: 211-218.

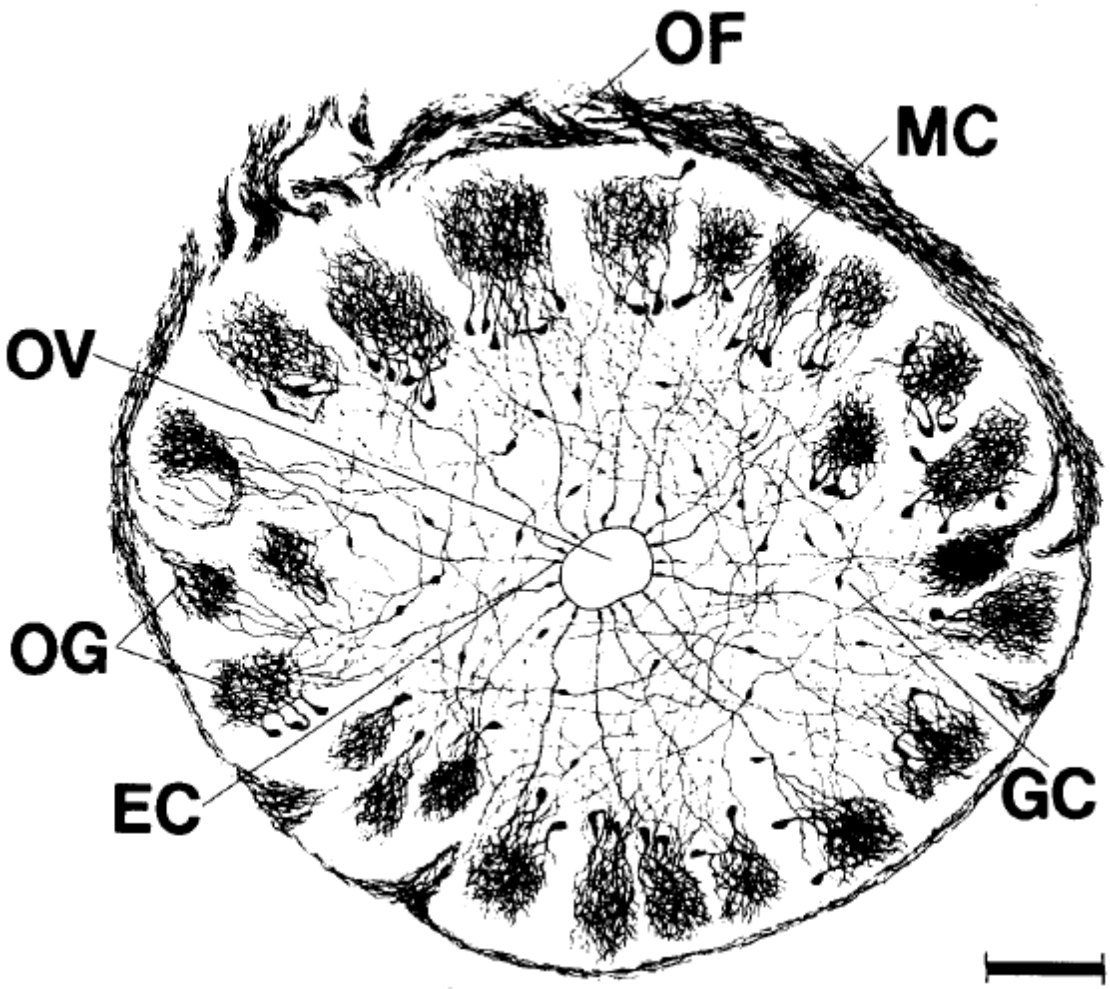


Figure 1.2. The lamprey olfactory bulb circuity. The exterior layer that contains the OSN's is called the layer of the olfactory fibers (OF). The next layer is called the olfactory glomerular layer (OG), which contains the mitral cells (MC). The third layer is the granule cell layer (GC), which contain granule cells and have projections to the glomerular layer. The fourth layer is called the ependymal cell layer (EC), which contain ependymal cells and also have projections into the glomerular layer. Illustration from Iwahori et al, 1987. Neuroscience research. Vol 5: 126-139.

suppression of neural activity. An important neurotransmitter in the brain of vertebrates that is frequently involved in neuromodulation is 5-HT. 5-HT has been linked to inhibitory and excitatory modulation of a wide variety of behaviours such as: feeding, sleep, moods, aggression, anxiety, social hierarchies, escape behaviour and body weight regulation (Best et al., 2010). For example, there was a significant reduction in food intake after intracerebroventricular injection of 5-HT in goldfish (De Pedro et al., 1997). In crayfish, 5-HT was shown to have an effect on enhancing aggression to achieve social status, while causing a decline in the escape ability of subordinate crayfish (Edwards et al., 1999). An interesting study by Hapiak et al. (2006) on the nematode, *Caenorhabditis elegans*, involved dual excitatory and inhibitory serotonergic inputs. In order to modulate nematode egg laying, a combination of both inhibitory and excitatory serotonergic inputs mediated by 5 different serotonergic receptors was required (Hapiak et al., 2006). 5-HT has also been linked to stress in mammals and fish. When a stressful condition arises, researchers have found that there is an increase in the metabolism of 5-HT in the brain (Khan and Hasan, 1984; Khan, 1986; Winberg and Nilsson, 1993; Khan et al., 1997). One example of stress was shown in the juvenile carp when levels of 5-HT increased in the brain during times of thermal stress (De Boeck et al., 1996) indicating that 5-HT could be directly involved in the regulation of temperature. It is clear that 5-HT plays an important modulatory role in certain behaviours in a variety of organisms and that the different 5-HT receptors play a part in regulating this modulation.

One of the most common examples of 5-HT modulation enhancing a neural response has been shown in the *Aplysia*. In this invertebrate model species, the gill-withdrawal reflex is subjected to short term sensitization by 5-HT facilitating interneurons. Sensitization occurs when a stimulus applied to one pathway causes a change in the reflex strength of another pathway. In

the *Aplysia*, a noxious stimuli is applied to the tail, which causes an excitation of 5-HT facilitating interneurons. The 5-HT binds to receptors on the presynaptic sensory neuron, which causes an increase the level of the second messenger, cyclic adenosine monophosphate (cAMP), activity in the terminal of the sensory neuron, which leads to closing of the K^+ channels and a prolonged period of Ca^+ influx into the cell during the action potential. Ultimately, this enhances the gill withdrawal reflex and increases the amount of 5-HT available for the motor neuron in the synaptic cleft (Kandell 2000) (Figure 1.3).

The majority of 5-HT in the body of mammals is found in enterochromaffin cells of gastrointestinal (GI) tract in the intestine and the remainder is found in neurons located in the raphe nuclei of the brainstem centered around the reticular formation (Blanco-Centurion 2001). Over evolutionary time, the serotonergic system in vertebrates, such as the lamprey and bony-fish, showed a transition from populations located in the forebrain regions to brainstem regions (Parent et al., 1984; Abalo et al., 2000).

1.3.1 Studies of 5-HT modulation by bath application

There have been numerous studies testing the modulatory effects caused by 5-HT through bath application, most of which caused inhibitory effects. Bulbring et al. (1958) tested the effect of 5-HT on peristalsis in the rabbit. When they added 5-HT intrinsically, they found that it caused peristalsis to be stimulated. However, when they added 5-HT externally by bath application, they discovered that it caused peristalsis to be inhibited (Bülbring et al., 1958). In the zebrafish, 5-HT applied through bath application was discovered to inhibit the locomotor rhythm of motor neurons in the spinal cord (Gabriel et al., 2009). The effects of 5-HT on rat dentate gyrus neurons, part of the hippocampus formation, by intracellular recordings in brain slices were examined by Piguet et al. (1994). They found that through bath application of 5-HT

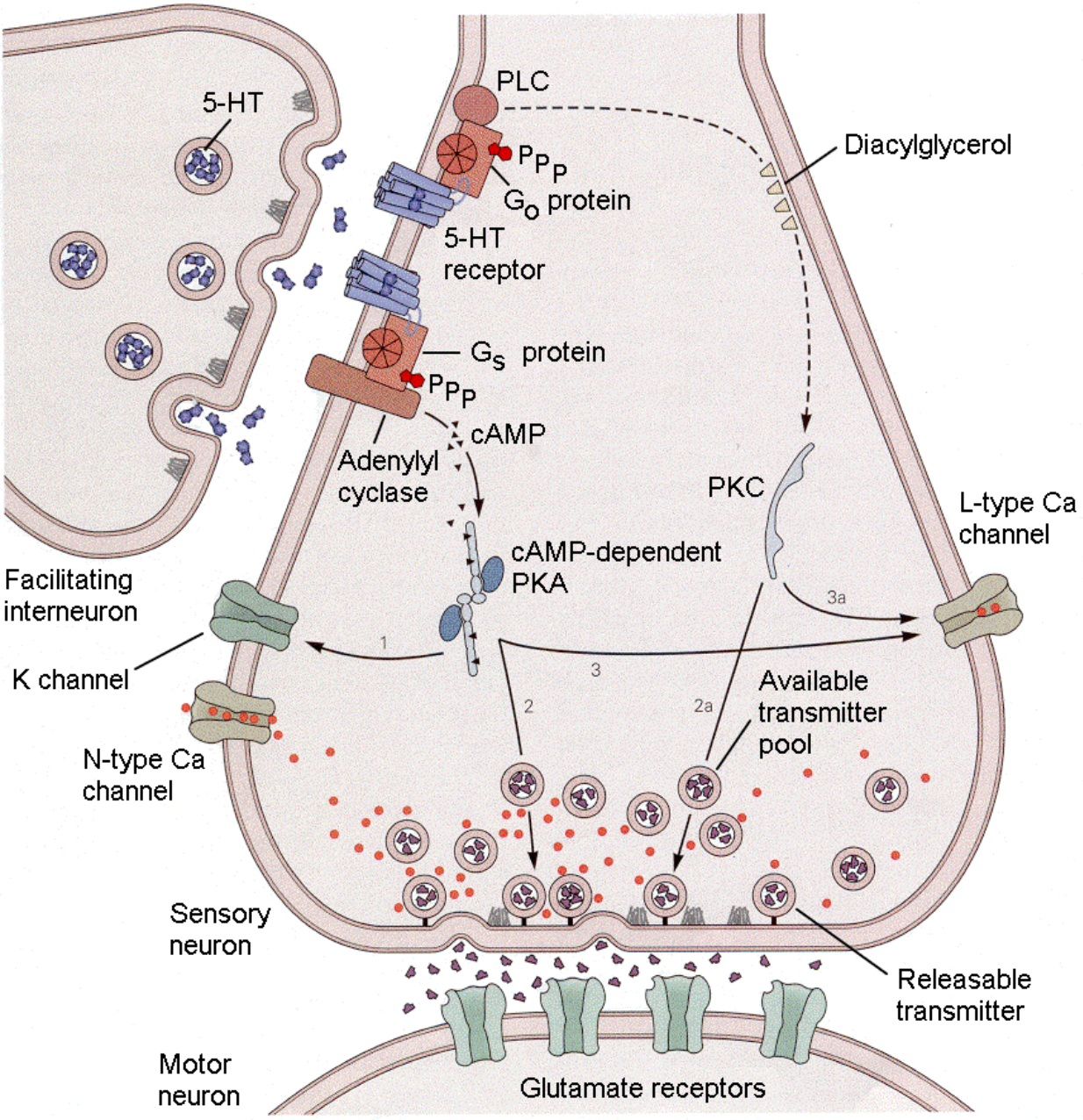


Figure 1.3. Facilitation of the sensory neuron by the 5-HT interneuron. A stimuli excites the 5-HT interneuron causing 5-HT to be released and bind to G-protein coupled receptors on the sensory neuron. This results in the activation of the second messenger, adenylyl cyclase (AC). The AC increases the level of cyclic adenosine monophosphate (cAMP) in the terminal of the sensory neuron by converting adenosine triphosphate (ATP) to cAMP. As a result, cAMP activates cAMP-dependent protein kinase A. This causes the potassium (K) channel to be closed and prolongs the influx of calcium (Ca) during the action potential. Ultimately, this leads to a longer reflex period and an increased amount of 5-HT available for release into the synaptic cleft. Image from Kandel 2000. Principles of Neural Science 4th edition: 1250-1255.

and local application of 5-HT by pressure pipette, the membrane potential was hyperpolarized meaning that 5-HT had an inhibitory effect (Piguet et al., 1994). Interestingly, when examining the effect of 5-HT bath application on the subthalamic neurons of mouse brain slices, Stanford et al. (2005) discovered that 5-HT bath application caused inhibition and excitation on the same neuron at different times. Ultimately, they determined that the excitation was caused by the activation of the 5-HT2c and 5-HT4 receptors, while the inhibitory effects were seen when the 5-HT1a receptor was activated (Stanford et al., 2005). During current clamp recordings from the rat spinal motorneurons, 5-HT bath application caused a depolarization of the motorneurons meaning that 5-HT had an excitatory on the neuron firing (Takahashi et al., 1990). In the lamprey, application of 5-HT by bath application was found to cause an inhibitory effect on motor neurons in the spinal cord (Buchanan and Grillner 1991; Zhang et al., 1996) and was also shown to depress dorsal cell synaptic transmission leading to the spinal cord (El Manira et al., 1997). While most of these studies found that 5-HT bath application caused a suppression of neural activity, a few studies found that it caused an excitation of neural activity, while in a single study 5-HT caused suppression or enhancement depending on the method of delivery.

1.4. 5-HT modulation of the olfactory system

5-HT has been shown to have a modulatory effect on the olfactory system in a variety of animals. In mammals, the 5-HT fibers that originate in the raphe nuclei of the brainstem have projections to the glomerular layer in the OB where OSN's synapse onto mitral cells (McLean and Shipley 1987; McLean and Shipley 1987). The glomeruli are the initial circuitry that process odour information in the OB (Schoppa et al., 2003), so if 5-HT has modulatory effects in the glomeruli, it would modulate all of the ensuing odour coding in the olfactory system (Liu et al., 2011). A study done on the moth showed that modulation by 5-HT increased the amplitude of

odour-evoked responses in the neurons of the antennal lobe (Dacks et al., 2008), which is the primary olfactory processing center in the insect brain. However, in the mouse OB, activation of brainstem 5-HT neurons reduced the synaptic drive to mitral cells, which ultimately reduced the amplitude of olfactory responses and this inhibition was reversed when 5-HT was removed from the system (Petzold et al., 2009). In the rabbit, the application of 5-HT by microiontophoresis caused inhibition of mitral cell firing (Von Baumgarten et al., 1963; Bloom et al., 1964). In the rat OB, it was discovered through electrophysiological recordings on slice preparations, that 5-HT inhibited mitral cells by activating GABA-ergic local interneurons (Hardy et al., 2005).

5-HT fibers from the dorsal raphe nuclei (DRN) of the midbrain have been shown to heavily innervate glomeruli of the OB of rats (Moore et al., 1978; Areneda et al., 1980; McLean and Shipley 1987). This was also shown in the short tailed opossum (Philpot et al., 1994). The 5-HT fibers within the glomerular layer of the OB have 2 to 3 times greater density and are thicker than any other area that contains 5-HT in the OB (McLean and Shipley 1987). Therefore, it is no surprise that 5-HT modulation on the olfactory bulb is prominent in many vertebrate species and may occur in the sea lamprey.

1.5. 5-HT in the sea lamprey

One of the major roles that 5-HT plays in lamprey is the modulation of neural networks that control locomotion. There are 3 different systems of 5-HT in the lamprey spinal cord. The first are spinal 5-HT neurons that do not make any output snyapses (Christenson et al., 1990; Schotland et al., 1996) and communicate by transmitting signals from their dendrites. The second are dorsal column 5-HT axons that arise from dorsal root ganglion neurons (Harris-Warrick et al., 1985; Zhang et al., 1996). The third are descending 5-HT axons in the lateral and ventral columns that originate in the brainstem (Brodin et al., 1988; Zhang et al., 1996). In reticulospinal

motor neurons, 5-HT causes a decrease in the amplitude of the slow afterhyperpolarization (Grillner et al., 1985; Wallen et al., 1989; Buchanan and Grillner 1991; Grillner et al., 2003), which is the prolonged period of hyperpolarization in a neuron that follows an action potential, that ultimately causes an increase in the firing rate of ventral root neurons (Harris-Warrick et al., 1985; Christenson et al., 1989; Schwartz et al., 2005). This is accomplished by 5-HT inhibition of presynaptic transmission (Buchanan and Grillner 1991; Shupliakov et al., 1995) and postsynaptic inhibition of a calcium activated potassium current that is responsible for the sAHP (Schwartz et al., 2005). The result is an inhibition of neural activity and a decline in swimming behaviour (Grillner et al., 2003).

A large number of 5-HT receptor cells were discovered in the lamprey taste buds suggesting 5-HT could play a role in the transmission of gustatory signals or taste chemoreception processes (Barreiro-Iglesias et al., 2008). The presence of 5-HT was also found in the lamprey gills, head musculature and cranial nerve ganglia. Lampreys possess oxygen chemoreceptors in their gills to regulate their ventilation relative to the amount of oxygen in the water. Since 5-HT is found in the gills, it may have a modulatory effect on the oxygen chemoreceptors and ultimately the ventilation process in the lamprey (Barreiro-Iglesias et al., 2009). The distribution of 5-HT throughout the head musculature indicates that 5-HT may have a vital role in the muscular activity of sea lamprey and is a unique feature among vertebrates (Barreiro-Iglesias et al., 2009). It may also modulate viscerosensory or somatosensory stimuli from ganglion cells in the lamprey head (Barreiro-Iglesias et al., 2009). While 5-HT could have a modulatory effect on odour-evoked neural responses of the sea lamprey, it also has modulatory effects in the spinal cord and could be an important modulator in other areas of the lamprey.

1.5.1 The sea lamprey life cycle

The sea lamprey has distinct life history stages that include: the larval, parasitic and migratory adult phases (Hardisty and Potter 1971; Smith 1971). The larval remain buried in sediment in freshwater streams for approximately 3-18 years before they undergo metamorphosis and move into lakes or oceans to begin their feeding process (Li et al., 1995). They feed on other fish for 12-15 months (Potter et al., 1982; Bird et al., 1994) before migrating back to streams as adult lampreys to spawn.

1.5.2 Olfaction in the sea lamprey

Throughout their lifecycle, lampreys utilize olfactory cues to perform these basic behaviours of migrating, feeding and spawning (Dagfous et al., 2012). Lampreys locate their prey using amino acids released by the fish during the parasitic phase (Kleerekoper 1963). Adult lamprey detect a mixture of odours released by larval lamprey that includes: the bile acid, petromyzonol sulphate (PZS)(Li et al., 1995), petromyzonamine disulphate (PADS) and petromyzonolsterol disulphate (PSDS). Combined, these act as a migratory pheromone to detect suitable spawning streams. Once occupying the spawning streams, males release the sex pheromones 3-keto petromyzonol sulphate (3KPZS) and 3-keto allocholic acid (3KACA) that attract ovulating females to the male spawning nests (Li et al., 2002; Siefkes and Li, 2004; Johnson et al., 2009). All of these compounds have been shown to be detected by the olfactory system of the sea lamprey (Li et al., 1995; Sorensen and Li 1997; Bjerselius et al., 2000; Sorensen 2005). Ren et al. (2009) showed that the lamprey possess parallel olfactory processing pathways that consist of the main olfactory epithelium (MOE) and the accessory olfactory organ (AOO). When the lamprey is in the presence of an odour, olfactory sensory neurons in the MOE and the AOO will detect the odour molecules and project the odour information to the olfactory

bulb via the olfactory nerve (ON). In the lamprey OB, the dorsal, lateral, medial and ventral regions display chemotopy (Green 2012), which is the spatial patterning of bulbar activity related to odourant chemical features. The lateral OB responds specifically to amino acids, while the dorsal OB responds to bile acids, pheromones and amino acids (Green 2012). Chemotopy is also displayed in the channel catfish (Nikonov and Caprio, 2001) and mammals (Johnson et al., 1998; Johnson and Leon, 2007). In the sea lamprey, OSN's located throughout the MOE project to all regions of the OB, while OSNs located in the AOO project solely to the medial region of the OB. The medial region of the OB relays the information to higher brain structures such as the posterior tuberculum and mesencephalic locomotor region (MLR) that stimulate reticulospinal cells in the brainstem to initiate a motor response, while the non-medial regions project the information to the lateral pallium (Ren et al., 2009; Derjean et al., 2010). Since 5-HT fibers are distributed in the olfactory nerve and throughout different regions of the OB in the lamprey, they could have modulatory effects on different odours and odour-driven behaviours.

1.5.3 5-HT in the sea lamprey olfactory system

In the sea lamprey, there are 5-HT fibers throughout the primary olfactory pathway (Zielinski et al., 2000; Frontini et al., 2003) that could play a role in modulating olfactory responses (Figure 1.4). Some 5-HT cell bodies are located in the lamina propria (LP) located caudal to the olfactory epithelium (Figure 1.5) while none were found to originate in the OB (Zielinski et al., 2000). The 5-HT fibers spanned parallel along the ON from the lamina propria to the OB (Figure 1.6). Some of the 5-HT fibers terminated at the junction of the OB, while others continued into the OB. Those that entered were found in the dorsal OB and travelled along the olfactory nerve layer and others formed small entanglement of fibers adjacent to the ON (Zielinski et al., 2000). Further research on the distribution of 5-HT in the sea lamprey olfactory

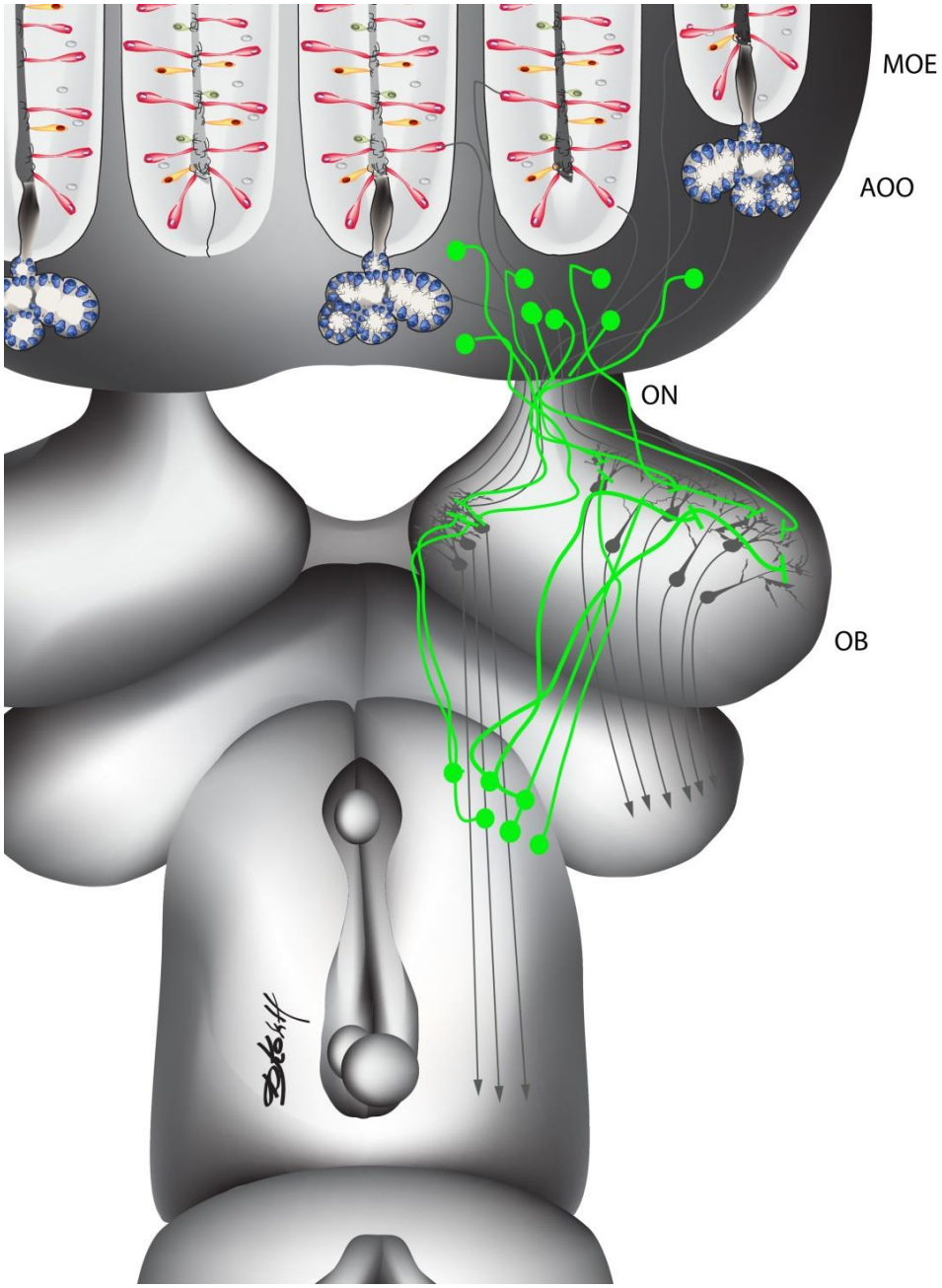


Figure 1.4. 5-HT in the primary olfactory pathway of the sea lamprey. When the lamprey is in the presence of an odour, olfactory sensory neurons (OSN's) in the main olfactory epithelium (MOE) or accessory olfactory organ (AOO) will respond to the odour molecules. The OSNs (grey) will project the information to all regions of the olfactory bulb (OB), which then relays the information to higher brain structures. 5-HT cell bodies (green) originate caudal to the MOE. The 5-HT fibers (green) run parallel to the OSN's along the olfactory nerve (ON) and synapse onto mitral cells in the dorsal, lateral and medial regions of the OB, while some 5-HT fibers stop in the ON and do not enter the OB. There are also 5-HT fibers that innervate the OB from 5-HT neurons that originate in the dorsal raphe nucleus in the midbrain. Image modified from Ren et al. (2000).

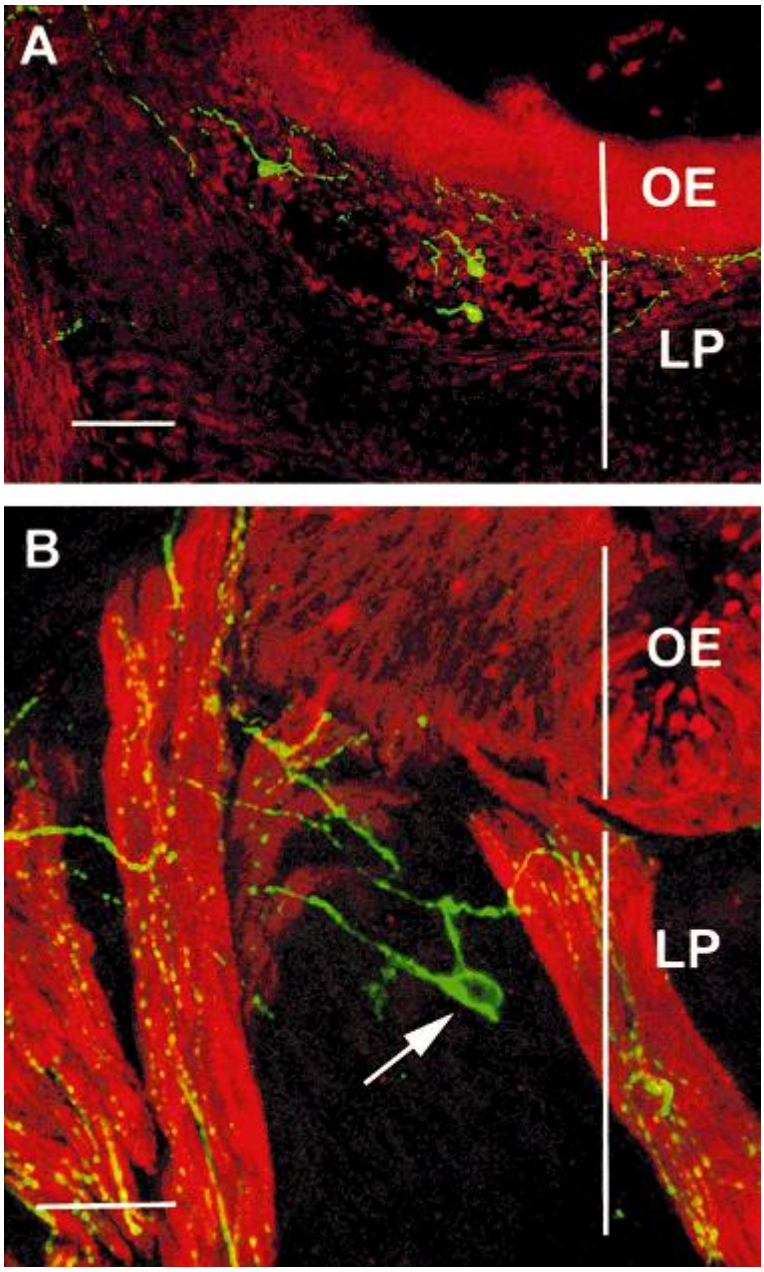


Figure 1.5. The origin of 5-HT cell bodies (green) in the lamprey primary olfactory pathway. **A:** In this particular section, there are 3 5-HT cell bodies in the lamina propria (LP) caudal to the olfactory epithelium. **B:** In this section, the 5-HT cell body (white arrow) is found between clusters of olfactory axons with two prominent processes shown stretching out of it. Image from Zielinski et al. (2000).

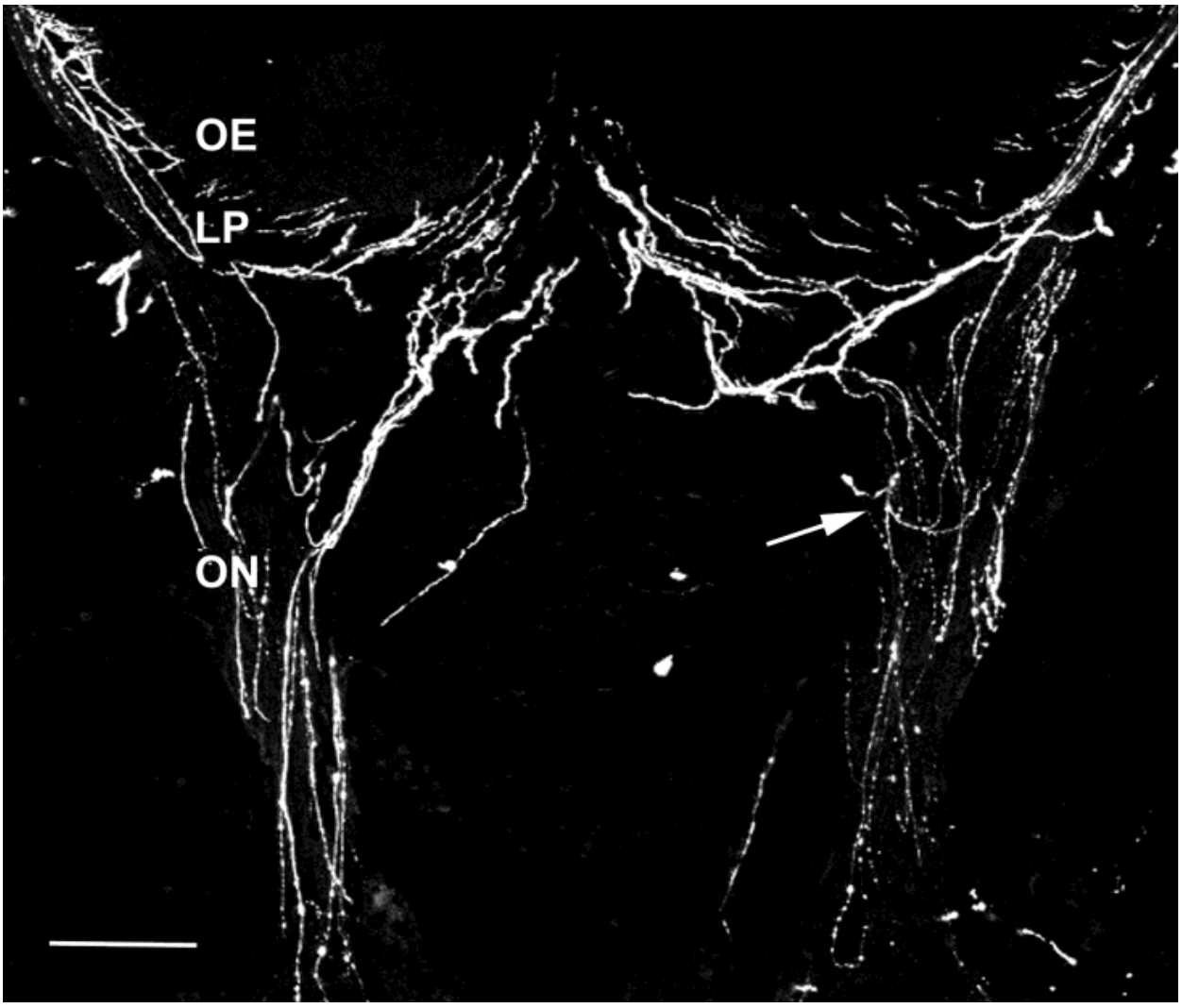


Figure 1.6. The distribution of 5-HT fibers (white) in the primary olfactory system of the sea lamprey. The fibers project caudal along the olfactory nerve (ON) from the lamina propria (LP) to the olfactory bulb. Image from Zielinski et al. (2000).

bulb found that 5-HT fibers were located in the dorsal, lateral and medial regions of the OB (Figure 1.7), but not in the ventral region (Frontini et al., 2003). In addition, there are 5-HT fibers from the dorsal raphe nuclei in the midbrain that enter the lamprey OB.

5-HT in the olfactory bulb of other vertebrates has been shown to play a role in olfactory learning. In 1 week old rat pups, depletion of 5-HT in the olfactory bulb prevented the pups from acquiring odour preferences (Langdon et al., 1997). McLean et al. (1993) showed that the innervation of 5-HT fibers in the OB are required for expression and acquisition of olfactory based learned behaviour in the neonate rat. 5-HT in the olfactory bulb has also been linked to a role in olfactory learning in the short-nosed fruit bat, *Cynopterus sphinx* (Ganesh et al., 2010). These bats find stationary food using olfactory cues in environments stricken with obstacles (Ganesh et al., 2010). When doing behavioural tests, it was shown that a depletion of 5-HT from the OB of the bat resulted in failure to learn novel odours and a failure to remember odours they were previously subjected to (Ganesh et al., 2010).

1.6 The 5-HT receptors

The family of 5-HT receptors is composed of seven distinct receptors, 5-HT1 through 5-HT7 and many of those are divided into subpopulations. The 5-HT1 receptor is composed of the 5-HT1a, 5-HT1b, 5-HT1d, 5-HT1e and 5-HT1f receptor subtypes. The 5-HT1a, 1d, 1e and 1f receptors are all found mainly in the central nervous system, while the 1b receptor is found both in the central nervous system and some peripheral nerves (Hoyer et al., 1994). The 1a receptor causes hyperpolarization of neurons (Hoyer et al., 1994; Barnes et al., 1999), while the 1b and 1d cause neurotransmitter release to be inhibited and 1e and 1f cause adenylyl cyclase to be inhibited (Hoyer et al., 1994). The 5-HT1b receptor and the 5-HT1d receptor are located on the terminals of 5-HT neurons. Interestingly, they are also located on the terminal of modulatory

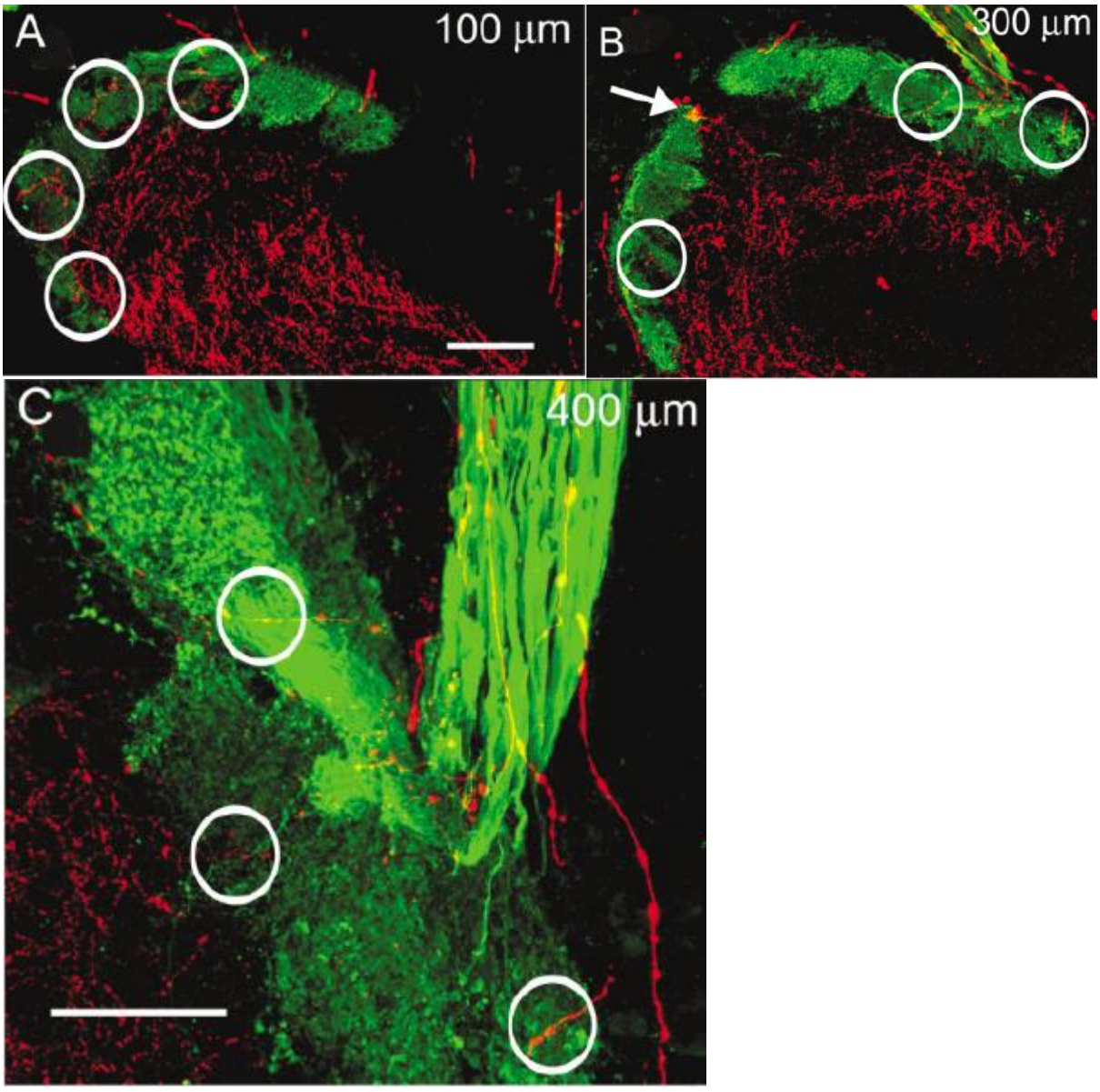


Figure 1.7. The distribution of 5-HT fibers (red) in the olfactory bulb (OB) of the sea lamprey. $GS1B_4$ is labelled green and the yellow represents areas of overlap between the 5-HT fibers and axons of olfactory sensory neurons (OSNs) indicated by a white circle. **A:** 5-HT fibers in the dorsal region of the OB. There are 4 regions that show close proximity between 5-HT fibers and OSN's. **B:** 5-HT fibers located in the lateral OB with 3 regions of close proximity. **C:** 5-HT fibers found in the medial region of the OB that also display 3 regions of close proximity. Images from Frontini et al. (2000).

neurons such as GABAergic, glutamatergic and dopaminergic neurons and act to inhibit these neurons from releasing neurotransmitters (Bockaert et al., 1996).

The 5-HT2 receptor is composed of the 5-HT2a, 5-HT2b and 5-HT2c receptor subtypes. The 5-HT2a receptor subtype is found in the CNS, platelets in the lung, the GI tract and the vascular smooth muscle (Hoyer et al., 1994). The 5-HT2b is mainly found in the PNS while 5-HT2c receptors are found in the CNS (Hoyer et al., 1994). All of these receptor subtypes have been linked to an increase in phosphoinositide metabolism (Bradley et al., 1986). The 5-HT2a receptor has been shown to cause excitatory effects, such as during intracellular electrophysiological recordings from interneurons in the rat brain (Barnes et al., 1999).

The 5-HT3 receptor is known to cause an excitatory effect in some of the central and peripheral nervous system neurons such as mediating the neuronal reflex effects caused by 5-HT in the periphery (Hoyer et al., 1994). The application of 5-HT3 antagonists have been linked to a decrease in anxiety related behaviour (Barnes 1999) and an increase in cognitive processes (Bentley and Barnes 1995).

The 5-HT4 receptor is found in the urinary bladder, the heart, the CNS and the GI tract (Hoyer et al., 1994). The 5-HT4 receptor is positively linked to adenylyl cyclase (Hoyer et al., 1994) meaning that it would increase the production of cAMP in CNS neurons, which would result in excitation of those neurons. It also causes the activation of acetylcholine in the gut (Hoyer et al., 1994; Barnes 1999).

The 5-HT5 receptor is the least understood of all the 5-HT receptors. It is composed of the 5-HT5a and 5-HT5b receptor subtypes, both of which are limited to distribution in the CNS. The 5-HT5a receptor has been shown to act in a similar manner as the 5-HT1a receptor in which

it will couple to G-proteins wherein the activity of adenylyl cyclase will be inhibited resulting in a inhibition of cell firing (Nelson et al., 2004).

The 5-HT6 and 5-HT7 receptors are very similar in that they are both located in the CNS and they act to increase the production of adenylyl cyclase (Hoyer et al., 1994). Ultimately, this will cause an increase in cAMP levels within the cell, which will cause excitability of neuron firing.

1.6.1 The 5-HT1a receptor

Recently, Barreiro-Iglesias et al. (2012) showed the expression of the 5-HT1a receptor throughout the lamprey OB. 5-HT1a receptor binding sites are found distributed highly in the dorsal raphe nuclei and in limbic brain areas and in the spinal cord (Barnes et al., 1999). Within the raphe nuclei and forebrain regions, they are located on 5-HT neurons themselves and post-synaptic to 5-HT neurons (Radja et al., 1991). Electrophysiological studies involving the 5-HT1a receptor have determined that its activation causes neuronal hyperpolarization (Hoyer et al., 1994; Barnes et al., 1999). It was also determined that the application of 5-HT1a antagonists reversed the inhibitory effect on cell firing caused by 5-HT and 5-HT1a agonists (Craven et al., 1994; Gartside et al., 1995). At the neuronal level, the 5-HT1a receptor has been shown to be an important factor in synaptic physiology and plasticity (Polter et al., 2010). At the behavioural level, the 5-HT1a receptor has been shown to have roles in anxiety related behaviours (Zhuang et al., 1999), depression and learning and memory (Polter et al., 2010). When applying 5-HT1a agonists to rodents during social interaction and maze tests, it was discovered that the agonists decreased anxiety (Dunn et al., 1989) suggesting that 5-HT decreases anxiety in stressful conditions. Further supporting this notion, knockouts of the 5-HT1a receptor performed in mice caused elevated anxiety-like behaviours when performing the maze test (Ramboz et al., 1998). 5-

HT1a receptors also play a role in mood related behaviours, in particular, depression. Delivery of 5-HT1a agonists caused a reduction in the depressive behaviour displayed during a forced swim test in rats (Kostowski et al., 1992; Wielend et al., 1990) and during a suppressed feeding test (Santarelli et al., 2008). The 5-HT1a receptor has also been shown to have an influence on depression in humans. In the post-mortem brains of depressed subjects, there was a clear reduction of 5-HT1a receptors in critical areas of the brain (Savitz et al., 2009). The 5-HT1a receptor has also been linked to passive avoidance learning in a number of studies (Polter et al., 2010). This occurs when animals are taught to avoid natural response behaviours to stimuli that cause stress by relating those stimuli to a shock. Application of 5-HT1a antagonists caused an increase in learning in mice (Madjid et al., 2010), which suggests that the 5-HT1a receptor could have an influence on inhibitory learning.

The 5-HT1a receptor is an inhibitory G-protein coupled receptor (Hoyer et al., 1994). When 5-HT binds to the 5-HT1a receptor on the cell membrane, it will cause the G-protein subunits γ, α and β to dissociate (Polter et al., 2010). The α subunit will inhibit the production of adenylyl cyclase (AC), which thereby decreases the production of the second messenger, cyclic adenosine monophosphate (cAMP). This will prevent cAMP from binding to and activating cyclic nucleotide gated (CNG) channels on the membrane meaning that the influx of positive ions into the cell is impeded (Polter et al., 2010). The β and γ subunits will activate G-protein coupled inward rectifying potassium (GIRK) channels on the cell membrane, which causes potassium ions to flow out of the cell. Ultimately, this causes an increase of negative ions inside the cell, which results in hyperpolarization of the cell (Polter et al., 2010) (Figure 1.8). The action of a 5-HT1a antagonist, such as spiperone or s(-)-uh-301, will reverse the inhibitory effect on cell depolarization caused

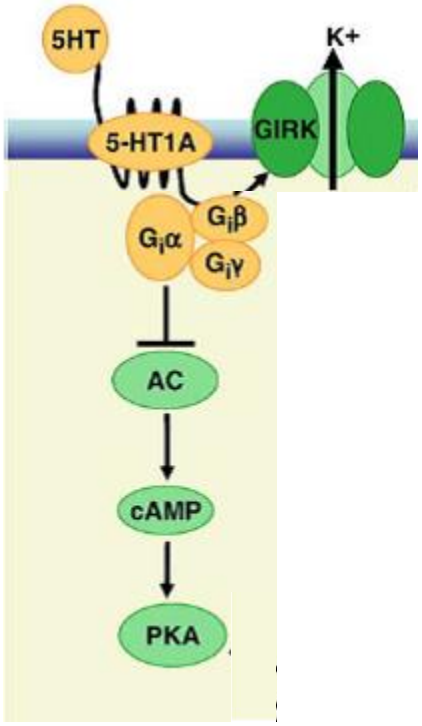


Figure 1.8. The 5-HT1a receptor signal transduction pathway. When 5-HT binds to the G-protein coupled 5-HT1a receptor, it exchanges GDP for GTP, which activates the G-protein subunits causing them to dissociate. Activation of the alpha subunit will cause the subunit to bind to and inhibit the production of adenylyl cyclase (AC), which results in a decreased production of the second messenger cyclic adenosine monophosphate (cAMP) and PKA activity. This prevents cAMP from activating cyclic nucleotide gated (CNG) channels for the influx of positive ions. The binding of 5-HT will also cause the beta and gamma subunits to activate G-protein coupled inward rectifying potassium (GIRK) channels. This causes potassium ions to flow out of the cell making the interior more negative and thereby inhibiting cell depolarization. Image modified from Polter et al. (2010) Cellular Signalling 22 (2010): 1406-1412.

by 5-HT. The antagonists bind to the 5-HT1a receptor on the cell membrane, which prevents the activation and dissociation of the G-protein subunits. The α subunit is then incapable of inhibiting AC, which means that cAMP will be produced at normal levels. The cAMP will now bind to CNG channels on the cell membrane, which will allow the influx of positive ions into the cell (Polter et al., 2010). The β and γ subunits will no longer be activate the GIRK channels so they will remain closed, thus reducing outward potassium conductance. This results in maintained positive ions inside the cell and therefore a lack of cell membrane repolarization (Polter et al., 2010) (Figure 1.9).

Two 5-HT1a antagonists used in previous sea lamprey research are spiperone hydrochloride and S(-)-5-fluoro-8-hydroxy-dipropylaminotetralin hydrochloride (S(-)-UH-301) (Grillner et al., 1995; Dacus 2000; Grillner et al., 2000; Martin et al., 2002; Grillner et al., 1995; El Manira et al., 1997). Spiperone (Grillner et al., 1995; Dacus 2000; Grillner et al., 2000; Martin et al., 2002) and s(-)-uh-301 (Grillner et al., 1995; El Manira et al., 1997) were shown to increase the amplitude of the slow afterhyperpolarization after action potentials in the lamprey, which had previously been reduced by 5-HT. Spiperone acts as a 5-HT1a and 5-HT2 receptor antagonist (Hoyer et al., 1994; Grillner et al., 1995; Barnes et al., 1999). It has also been demonstrated to act as a dopamine 1 and dopamine 2 receptor antagonist (Seeman et al., 1994). However, s (-)-uh-301 is highly specific to binding at the 5-HT1a receptor site. It was shown to have a 70-fold greater selectivity for the 5-HT1a recognition site over all of the other 5-HT receptor sites (Moreau et al., 1992). In contrast, spiperone has an 80-fold higher affinity for the 5-HT2a receptor binding site than the 5-HT1a receptor binding site (Hoyer et al., 1994). Since the 5-HT1a receptor is distributed throughout the lamprey OB (Barreiro-Iglesias et al., 2012) and

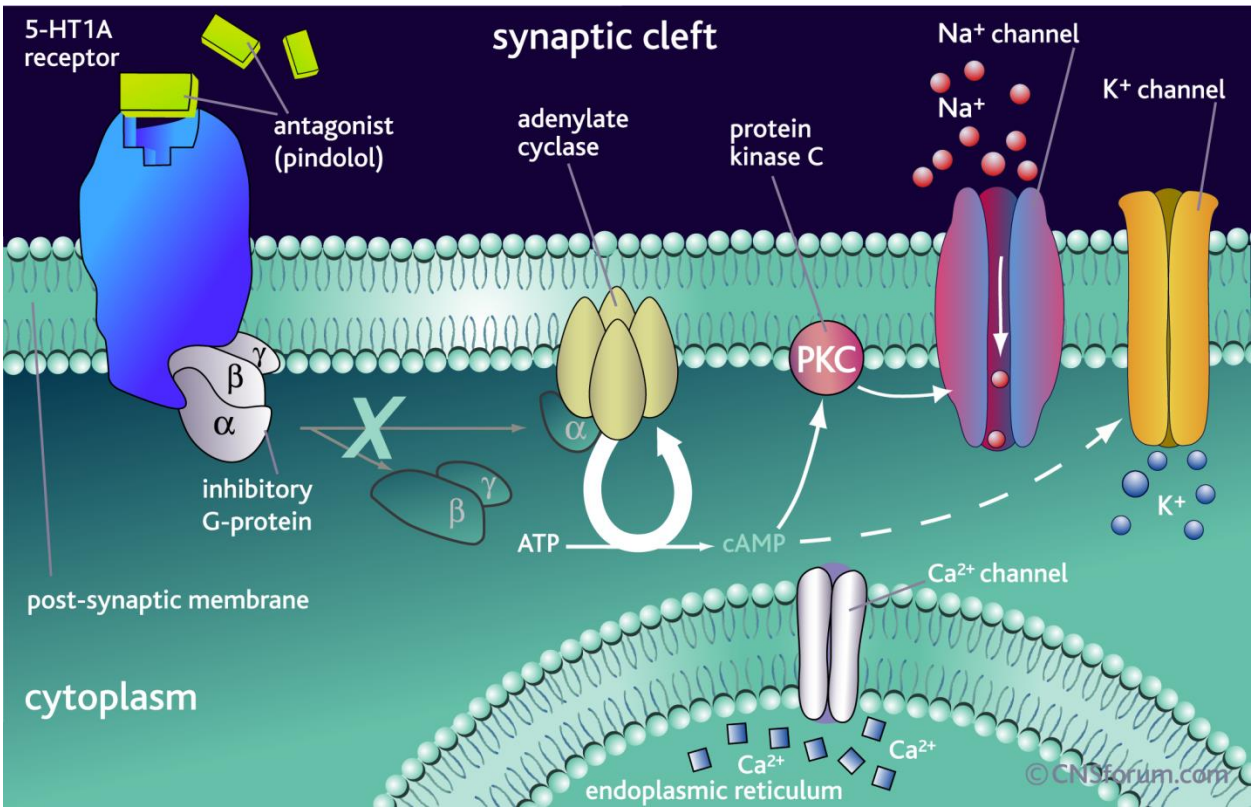


Figure 1.9. Action of 5-HT1a antagonists on the 5-HT signal transduction pathway. When a 5-HT1a antagonist (pindolol in this example) binds to the 5-HT1a receptor, it masks the inhibitory effects caused by 5-HT. The alpha subunit of the G-protein coupled receptor will not inhibit the production of adenylyl cyclase (AC) meaning that AC will convert adenosine triphosphate (ATP) to cyclic adenosine monophosphate (cAMP). cAMP will activate cyclic nucleotide gated (CNG) channels causing an influx of positive ions to the cell. The beta and gamma subunits will not activate the G-protein coupled inward rectifying (GIRK) channels meaning that the channels will remain closed and positive potassium ions will not leave the cell. The result is depolarization of the cell. Image from In: Pharmacology, 4th edition. Rang HP, Dale MM and Ritter JM. Edinburgh, UK: Harcourt Publishers Ltd, 2001:2–46.

spiperone and s(-)-uh-301 were shown act as 5-HT1a antagonists in the lamprey spinal cord, they should be a good choice for investigating 5-HT1a function in the OB. Two antagonists were used to ensure that the effects of the antagonists were consistent in blocking the activity of the 5-HT1a receptor in the OB.

1.7 Overview of Thesis

Despite the extensive amount of 5-HT fibers found throughout the lamprey olfactory system, there have been no previous studies examining the possible modulatory effects of 5-HT on olfactory responses in the lamprey OB. The purpose of this thesis is to utilize electrophysiological recordings for determining if 5-HT has modulatory effects on odour responses in the OB of the sea lamprey. The dorsal and lateral regions of the OB were chosen for recording locations. The dorsal region of the OB has the highest density of 5-HT fibers in the lamprey (Zielinski et al., 2000; Frontini et al., 2003) and in order to examine if there were any spatial differences in 5-HT modulation, the lateral region, also shown to contain 5-HT fibers (Frontini et al., 2003), was also chosen. Recordings were not taken from the medial region because the dorsal and lateral regions could be part of an indirect olfactory-motor pathway whereas the medial region is involved in a direct olfactory-motor pathway. In Experiment 1, the existence of 5-HT modulation of odour responses was probed using bath application to the lamprey brain-olfactory preparation. In Experiment 2, the 5-HT1a antagonists, spiperone and s(-)-uh-301, were picospritzed into the lamprey olfactory nerve and olfactory bulb while odours were being applied to the main olfactory epithelium. The 5-HT bath application was used in order to determine if there was any 5-HT modulation on odour responses, while the picospritzing method was used in an attempt to localize the application to 5-HT in the primary olfactory pathway and investigate if it would cause modulation of odour responses.

Based on the previous neural and behavioural inhibitory effects caused by 5-HT in the lamprey and other vertebrates discussed in this chapter, I predict that the 5-HT bath application will cause both amino acid responses in the lateral OB and TCA, pheromones and amino acids in the dorsal OB to be inhibited. Since spiperone and s(-)-uh-301 reversed the inhibitory effect caused by 5-HT in the lamprey spinal cord (refer to Section 1.7), I anticipate that picospritzing spiperone and s(-)-uh-301 into the olfactory nerve will mask the inhibitory effect caused by 5-HT and thereby cause excitatory effects on odour responses in both regions of the OB.

Chapter 2

Materials and Methods

2.1 Animal Collection

Every experiment was performed in accordance with the guidelines of the University of Windsor Animal Care Committee and the Canadian Council on Animal Care. The sea lampreys were collected in the wild. Metamorphic stage seven sea lamprey (transformers) were caught by electroshocking techniques from the Little Carp River in Baraga, Michigan in October 2011 and the Western Lake Superior tributaries in October 2012. Spawning adult sea lamprey were caught by trapping in the Cheboygan River in Cheboygan, Michigan and the St. Marys River in Sault Ste. Marie, Ontario between June 2012-July 2013. All lamprey collection was done in collaboration with United States Geological Survey Hammond Bay Biological Station in Millersburg, Michigan. The animals were transported to the Department of Biological Sciences at the University of Windsor and housed in an 8ºC cold room in tanks with recirculated dechlorinated water until used for experimentation.

2.2 Ex-vivo olfactory epithelium and olfactory bulb preparation

The animals were anesthetized using a solution of tricaine mesylate (MS-222, 150mg/L, Sigma-Aldrich, Oakville, ON, Canada) in dechlorinated water, adjusted to a pH of 7 with 5M NaOH. The lampreys were decapitated at the level of the 3rd branchiopore and the remaining tissue was placed in a dissection dish filled with chilled lamprey Ringer's solution for the entirety of the surgery. The lamprey Ringer's solution was composed of: 130 mM NaCl, 2.1 mM KCl, 2.6 mM $CaCl_2$, 1.8 mM MgCl2, 4 mM HEPES, 4 mM dextrose, 1 mM $NaHCO_3$. Ringer's was made fresh for every experiment and the pH was adjusted to 7.4 with 5M NaOH. The Ringer's was also chilled and oxygenated (95% oxygen and 5% carbon dioxide) throughout the experiment. The dissection began on the ventral side of the tissue where a cut was made along the gill pores and removed down to the level of the buccal cavity and the nasopharyngeal pouch.

The tissue was then flipped over to the dorsal side up where the skin, musculature and cartilage were removed to expose the brain, olfactory nerve and nasal cavities. Lastly, the *dura matter* was removed from the olfactory bulbs and olfactory nerves. The olfactory epithelium was kept in-tact in an attempt to maximize responses to olfactory stimuli. The Ringers solution in the dissection dish was continuously replaced to ensure that the tissue was always submerged in a chilled environment. This preparation consisted of the olfactory epithelium and the brain up to the rostral-most portion of the spinal cord.

The tissue was placed in a recording chamber that was continuously perfused with Ringer's solution at a flow rate of 1ml/min. The Ringer's solution was held at a constant temperature of 10ºC throughout the entirety of the experiment in order to mimic the lamprey's natural environment. A minimum of 1 h was given for recovery before any recording was done. The Ringer's diffused into the recording chamber near the caudal portion of the preparation and was removed at the rostral portion near the olfactory epithelium via suction. A constant drip of Ringer's solution was applied to the olfactory epithelium by an odour delivery system (Figure 2.1).

2.3 Extracellular odour-evoked neural recordings

For Experiment 1, 5-HT was applied over the brain through bath application, and recordings were taken from an ensemble of neurons by extracellular local field potentials (LFP's) from the surface of the dorsal (N=13) and lateral (N=7) regions of the olfactory bulb (Figure 2.2) in metamorphic lamprey (N=18). For Experiment 2, 5-HT1a receptor antagonists were picospritzed into the olfactory nerve and extracellular LFP's were recorded from the dorsal (N=20) and lateral (N=12) regions of the olfactory bulb in female (N=18) and male (N=7) spawning adult lamprey. Recordings testing odour responses were taken from either the dorsal or

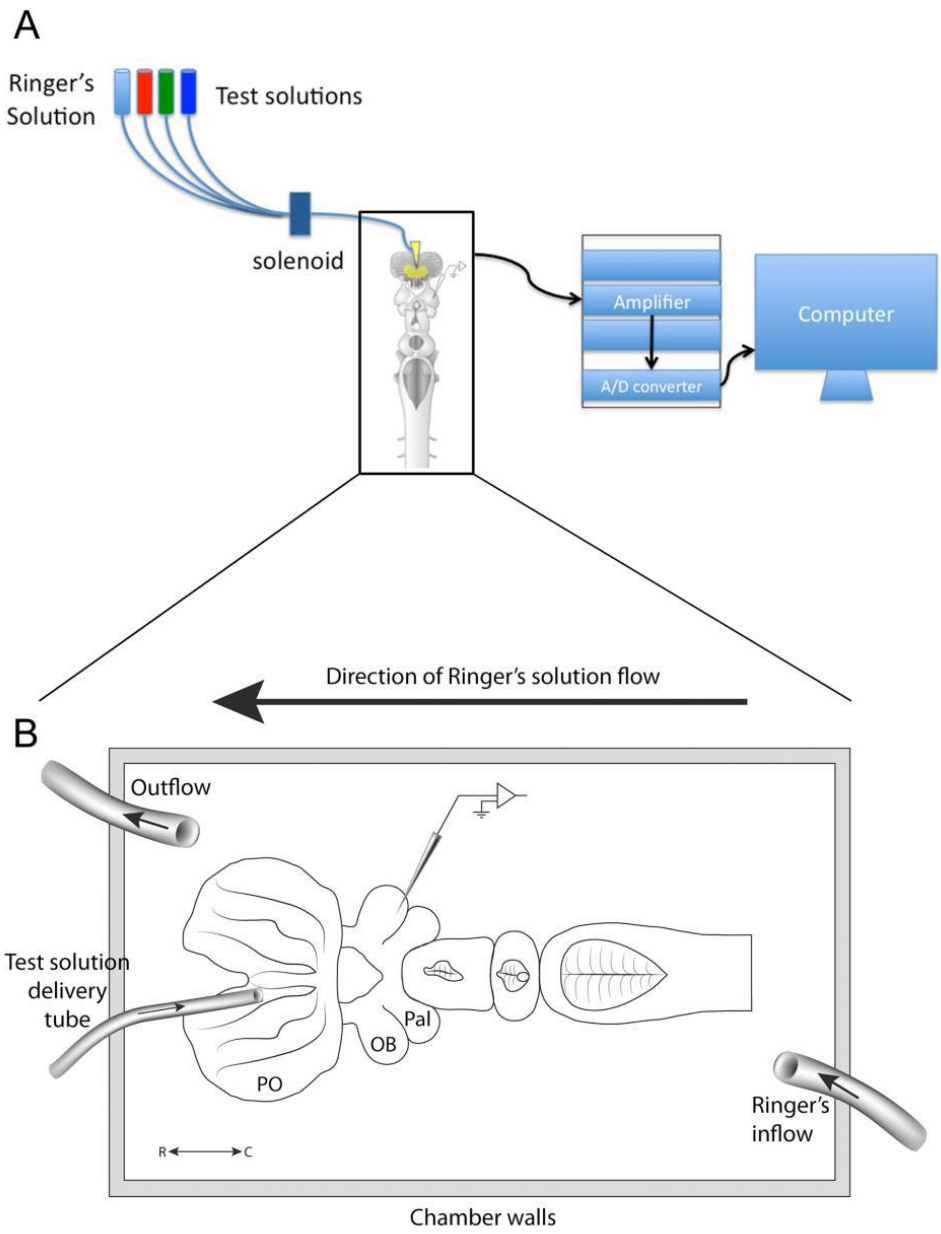


Figure 2.1. A diagram of the the odour delivery apparatus (A) and recording chamber (B) containing the sea lamprey olfactory organ/brain preparation. Chilled Ringer's solution flowed into the rear of the chamber, over the preparation and out via suction at the front of the chamber at a rate of 1ml/min. In order to help keep the Ringer's solution at a consistent temperature of 10ºC, the walls of the chamber contained cooled re-circulating anti-freeze. Chilled Ringer's and odourant solutions were applied to the peripheral olfactory organ (PO) through test solution delivery tubes. Extracellular LFP's to odourant test solutions were recorded from the olfactory bulb (OB). The signals were amplified, converted from analog to digital by the A/D converter (AD Instruments, Model ML-866, Colorado Springs, Colorado, United States) and sent to the computer for analysis. Caudal (C), Rostral (R), pallium (Pal). Illustration from Green 2012.

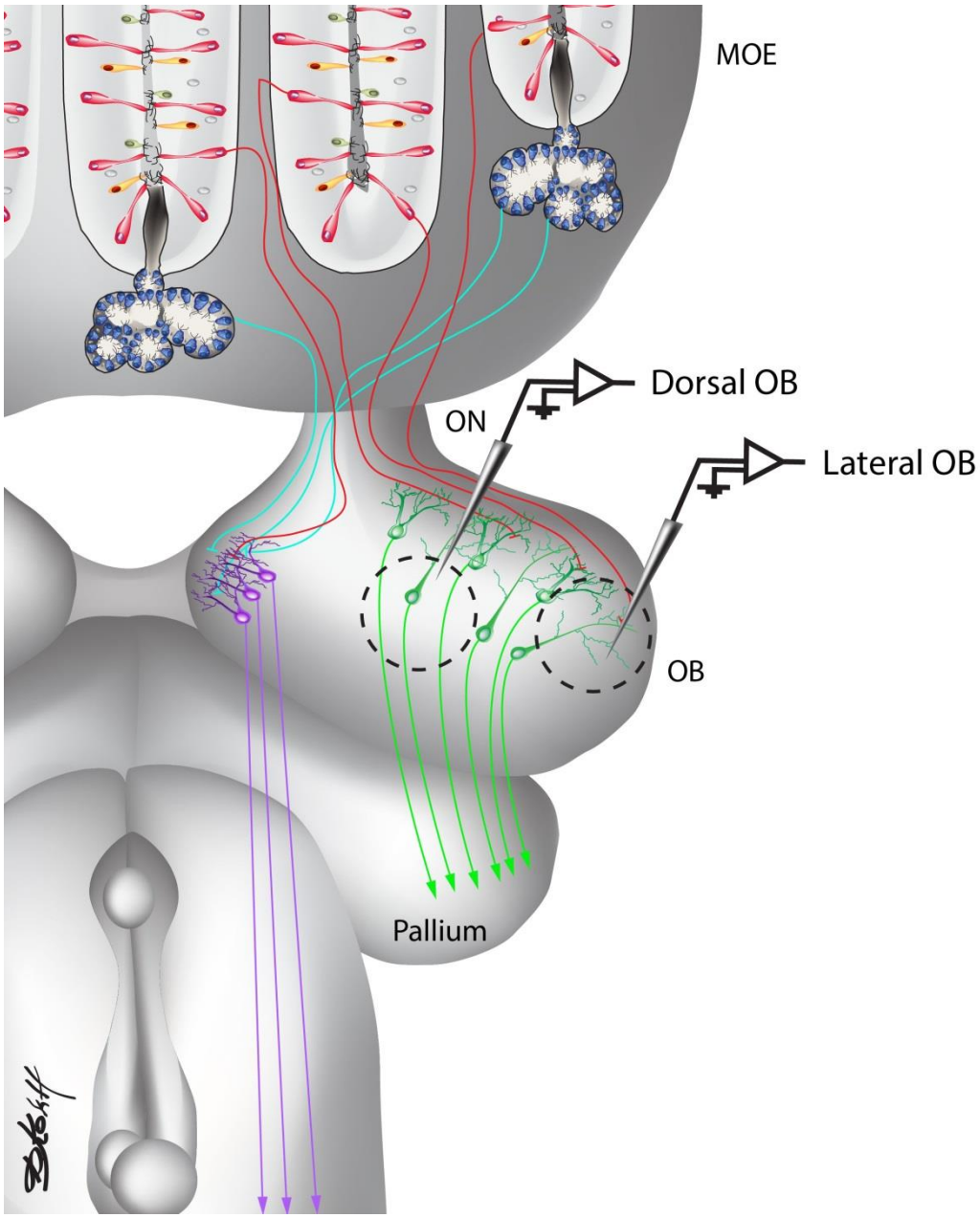


Figure 2.2. Recording locations of the extracellular LFP's in the olfactory bulb (OB). Hatched lines with an electrode denote the dorsal and lateral regions of the OB. Olfactory sensory neurons (red) from the main olfactory epithelium (MOE) project to the OB via the olfactory nerve (ON) and synapse onto mitral cells (green and purple) in the OB. When recording from the dorsal or lateral region of the OB, LFP recordings record from ensembles of olfactory sensory neurons and mitral cells located in their respective regions. The dorsal and lateral OB process that information and send it to the pallium. Illustration modified from Green 2012.

lateral region in some animals, while other recordings tested from both the dorsal and lateral region in the same animal. No differences were seen between male and female adult lamprey olfactory responses to the different odours so they were not separated during the data analysis. The recordings were done using glass micropipettes (World Precision Instruments, Inc., Sarasota, FL, 1.0mm) with a tip diameter of 5-10 µm that were filled with 2M sodium chloride (0.1 Mohm impedance). The micropipette was connected to a headstage by a silver conducting wire. The headstage-amplifier circuit was grounded and referenced by silver conducting wires that were placed in the recording bath and connected to the headstage. Using a micromanipulator (Narishige Inc.), the recording electrode was placed on the surface of the dorsal or lateral region of the olfactory bulb. During the recording, the LFP's were observed online using LabChart software (version 6.1.3, ADInstruments) in order to establish good signal to nose ratio and filtered between 1 Hz and 1kHz. The recordings were analyzed offline where they digitally filtered using LabChart. The signals were amplified 10,000 times during recording (model p511L amplifier, Grass Technologies Inc., West Warwick, RI, USA) and digitized at 10kHz (Powerlab 4/30, model ML866, ADInstruments, Colorado Springs, CO, USA) (Green 2012).

2.4 Preparation of odourant solutions and 5-HT for bath application

A stock solution of 10 mM taurocholic acid (T4009, Sigma-Aldrich) was made before every experiment in chilled lamprey Ringer's and kept on ice until just prior to experimentation when it was diluted in chilled Ringer's solution to concentrations of 1mM and 0.1mM by serial dilutions in chilled Ringers solution. Stock solutions of 10mM L-histidine (H-8000, Sigma-Aldrich) and 10mM L-arginine (H-5006, Sigma-Aldrich) were made in chilled lamprey Ringer's. These were kept on ice until just before experimentation when a mixture of 1mM L-arginine and 1mM L-histidine was made in Ringer's solution. After serial dilutions brought the mixture to

0.1mM, it was applied to the olfactory-brain preparation. The reputed pheromones (Yun et al., 2011; Li et al., 2002) that were used for the electrophysiology experiments were: 3-keto petromyzonol sulfate (KPZS), 3-keto allocholic acid (3KACA), petromyzonol sulfate (PZS), petromyzonamine disulfate (PADS) and petromyzonsterol disulfate (PSDS). The reproductive pheromones are 3KPZS and 3KACA while PSDS, PZS and PADS are migratory pheromones (Li et al., 1995; Sorensen and Li 1997; Bjerselius et al., 2000; Sorensen 2005). All of the pheromones were obtained from Dr. Weiming Li at Michigan State University in powder form (Pheromones received: May 2010 and June 25, 2013)(Batch IDs: PADS- 155-TNN-287D; 3KACA- 152-EJH-158-2; PSDS- 158-IDE-223-1; 3KPZS- 06 08 2006; PZS- 152-EJH-282-3). Stock solutions of each pheromone were made by restoring it in a 1:1 solution of ultra-pure water (18 MΩ.cm, Milli-Q reference system, Millipore, Billerica, MA, USA) : 1mg/mL high purity methanol (cat no. CABDH6048-4, VMR, Mississauga, ON, Canada). After the stocks were made, each pheromone was aliquoted out into 2mL screw top glass vials (cat no. 224881, Wheaton, Millville, NJ, USA) and were stored at -80ºC (Yun et al., 2011). Before every experiment, 0.01mM substocks of each pheromone were made up in Ringer's solution. Serial dilutions were used to make a mixed solution of 0.001mM 3KACA, 3KPZS, PADS, PSDS and PZS in Ringer's solution, which was delivered to the lamprey preparation. A solution of 1mM 5-HT (H9523, Sigma-Aldrich) was made in chilled lamprey Ringer's solution for every 5-HT bath application experiment and applied to the chamber at that concentration.

2.5 Experiment 1: Test of modulation of bulbar odour responses during bath application of 5-HT

A custom-made odour delivery apparatus delivered odours dissolved in Ringer's solution to the olfactory epithelium**.** Ringer's solution was continuously perfused over the olfactory epithelium by a gravity fed, valve-controlled odour delivery system. A three-way solenoid valve

that is electronically triggered through a computer was utilized in order to switch from the application of Ringer's solution to a particular odour in the olfactory epithelium (a design provided by Dr. D. Restrepo, U. of Colorado; Green 2012). The solenoid allowed for fast exchanging between the odours and background Ringer's without any changes in pressure to the nostrils or interruption in the flow. The use of a chiller allowed the odours and Ringer's solution delivered to the olfactory epithelium to be maintained at the same temperature as the chilled preparation. The odour was applied for 5 seconds during bath application experiments. Three minutes was allowed between odour applications when Ringer's would flow into the epithelium and the test odour would flow out of the system via suction rostrally. Previous research showed that 3 minutes between deliveries was sufficient time to prevent adaption to the odourants by the lamprey preparation and to show consistent olfactory responses to a repeated stimulus of odour solutions (Green 2012). Each odour was delivered between 3 times and a negative control (Ringer's solution) was tested at various times over every experiment. The Ringer's solution did not elicit olfactory responses from the lamprey preparation.

The 1mM 5-HT was applied to the recording chamber by a bath application via a peristaltic pump (model Minipuls-3, Mandel Scientific Company Inc., Guelph, Ontario, Canada). The tube that was running Ringers solution into the chamber through the peristaltic pump was removed from the Ringer's and placed into the solution of 1mM 5-HT, which began to fill the chamber (Figure 2.3). Time trials with the dye, Fast Blue (5%), determined that 15 minutes was the time required to displace Ringer's solution from the bath. Fast Blue did not elicit olfactory responses when applied to the olfactory epithelium. During each test for the effect of bath applied 5-HT on an odour response, there was first odour responses during a control by followed by odour responses during the 5-HT bath application and then during a Ringers washout period.

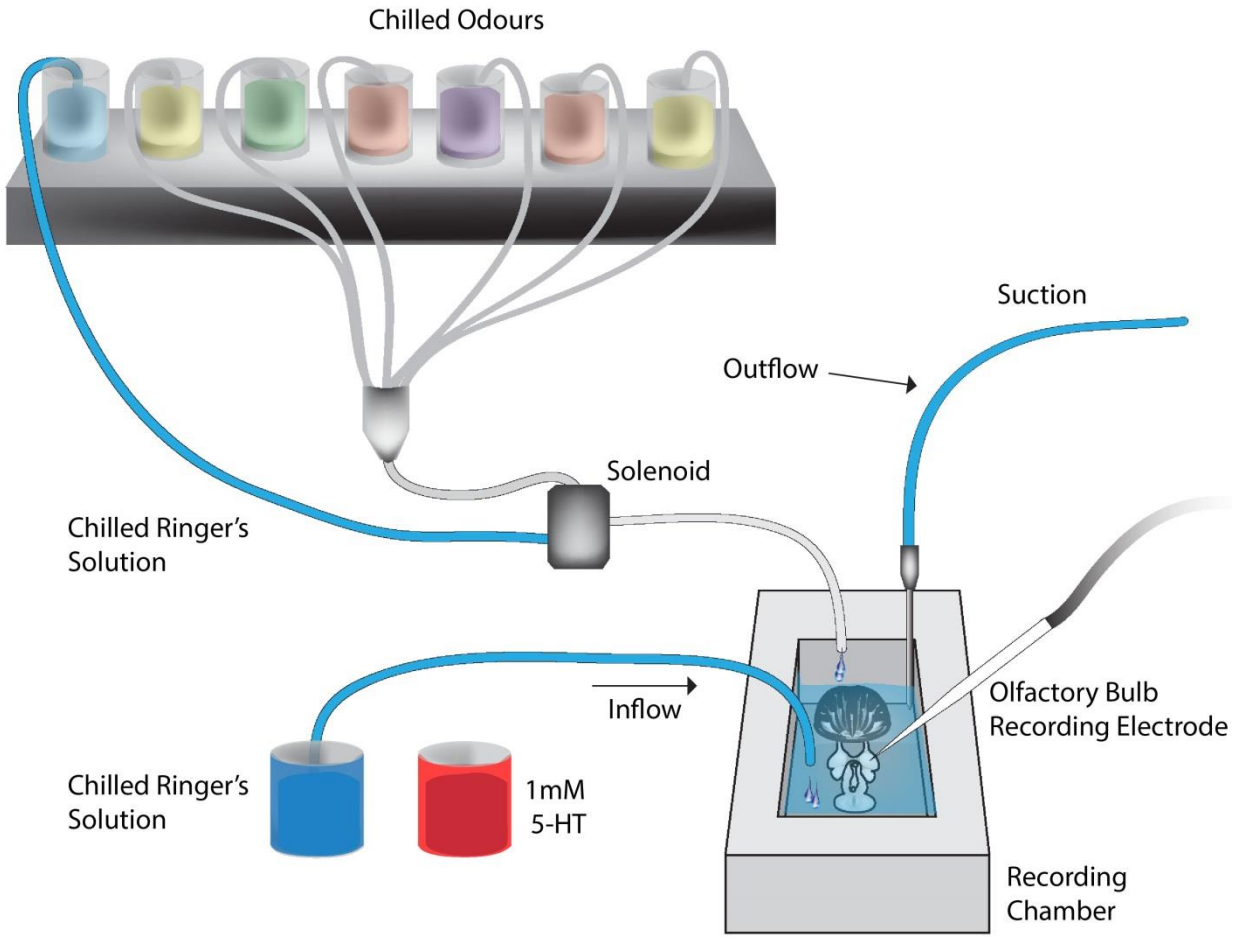
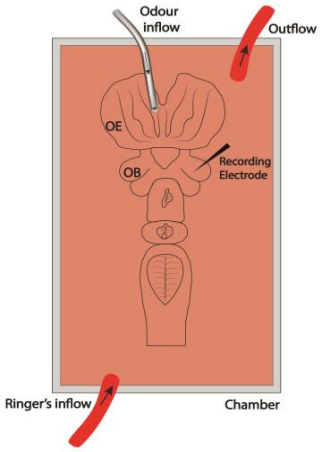
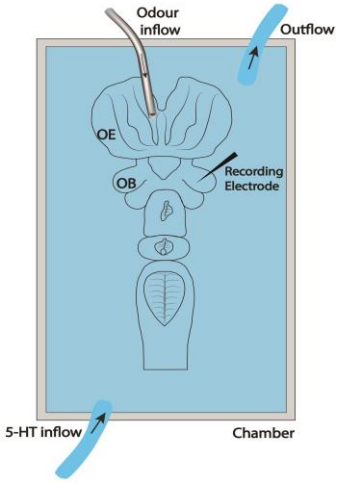


Figure 2.3. An illustration of the 5-HT bath application setup in experiment 1. The custom made odour delivery system delivered odours and a constant drip of Ringer's solution to the olfactory epithelium via a 3-way solenoid valve. The use of a chiller allowed for the odours to be cooled. The solenoid allowed for switching from the background Ringer's solution to an odour. While an odour was being applying to the olfactory epithelium, LFP's were being recorded from the olfactory bulb. The olfactory-brain preparation in the recording chamber was submerged in chilled Ringer's solution. When 5-HT effect were tested via bath application, the inflow tube was removed from the chilled Ringer's solution and placed into a solution of 1mM 5-HT in Ringers. This solution filled the chamber and displaced the Ringer's solution. Illustration modified from Green 2012.

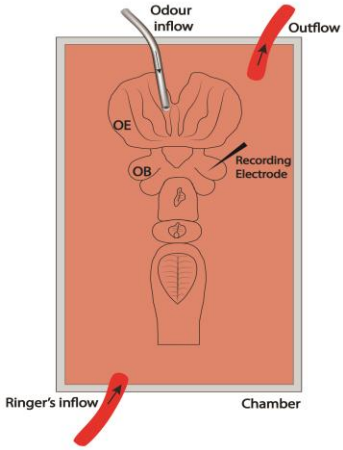
(A) Control (Odour)



(B) Odour + 5-HT (15 minute application)



(C) Washout (15 minutes)



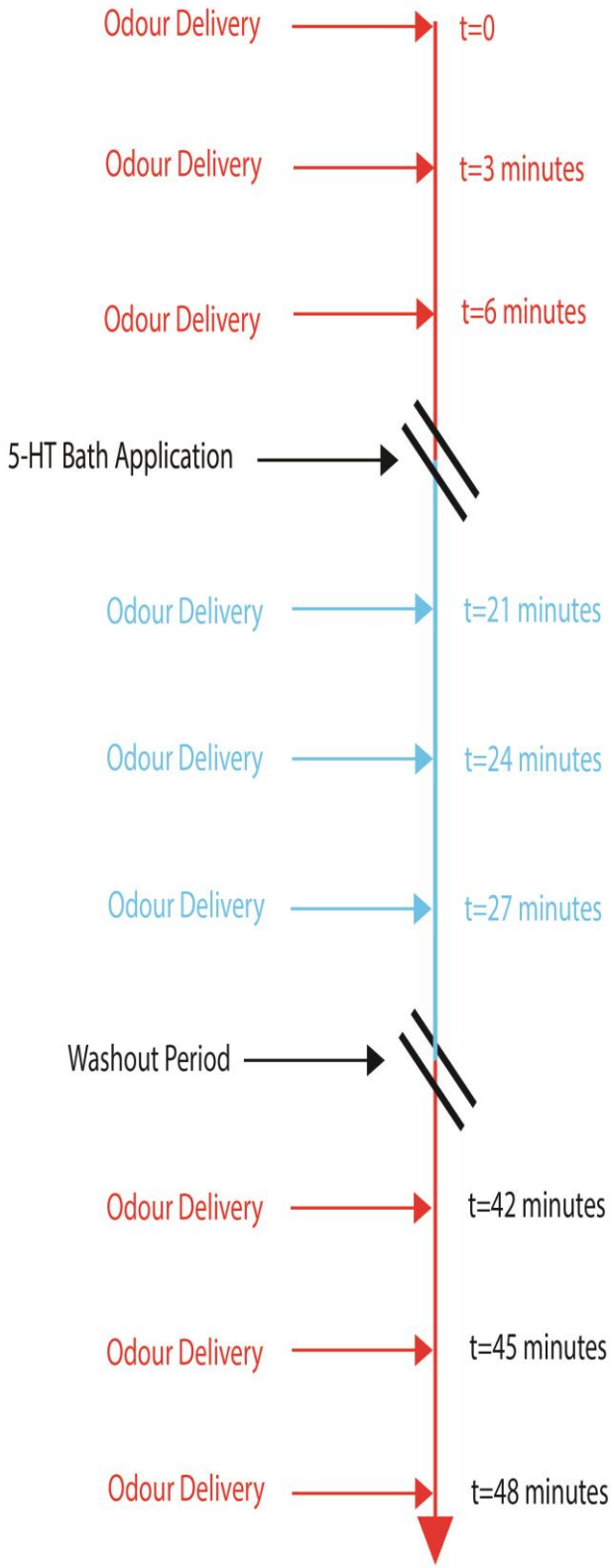


Figure 2.4. The time course for delivery of odourant test solutions in experiment 1. A: Odours were applied to the peripheral olfactory organ 3 times, with 3 minutes between deliveries, while recording LFP's from the dorsal or lateral region of the olfactory bulb (OB). B: It took 15 minutes to fill the chamber with 1mM 5-HT (blue). Once the bath had filled with 5-HT, the first odour application was delivered (t=21 minutes). After that, the same odour was delivered 2 times with 3 minutes between each application. C: A 15 minute washout period was given for the 5-HT to be removed from the chamber and replaced with Ringer's solution (red). The same odour was applied 3 times, with 3 minutes between deliveries, to examine the recovery from the 5-HT treatment. A-C: For the odour applications, background Ringer's solution was applied to the olfactory epithelium for 3 minutes, an odour was applied for 5 seconds and then back to background Ringers solution via the 3-way solenoid valve.

The control included 3 odour deliveries when the chamber was filled with Ringer's. Once 5-HT had completely filled the chamber, there were 3 odour deliveries. The timing of these odour deliveries in the 5-HT bath was based on the dye trials. The first odour delivery was applied to the lamprey preparation 15 minutes after 5-HT began to fill the bath and the next 2 odour deliveries were applied 3 minutes apart. Once the odour deliveries in the 5-HT bath were complete, a 15 minute washout period was given for Ringer's solution to displace 5-HT in the chamber. The washout period involved 3 odour deliveries after the 5-HT had been removed from the chamber (Figure 2.4).

2.6 Preparation of odourant solutions, 5-HT and 5-HT1a antagonists for picospritzing

Taurocholic acid, the mixture of amino acids and the mixture of pheromones were prepared for the picospritzing experiments in the same method as previously described (see section 2.2.4). Before every experiment, a stock solution of 1mM 5-HT was made in chilled lamprey Ringer's solution. The stock solution was diluted to 5µM 5-HT in Ringer's solution prior to delivery. A 100mM stock solution of the 5-HT1a antagonist, spiperone hydrochloride (sc-204293, Santa Cruz Biotechnology, Dallas, TX, United States), was made in dimethyl sulfoxide (DMSO)(D-6400, ACP chemicals, Toronto, ON, Canada). Once the stock was prepared, 100µL aliquots were stored in disposable scintillation vials (Z190527, Sigma Aldrich) at -20ºC. On the day of experimentation, one of 100mM aliquots was diluted to 10µM spiperone hydrochloride in chilled Ringer's solution by serial dilutions. A 1mM stock solution of the 5-HT1a antagonist, S(-)-UH-301 hydrochloride (S(-)-5-fluoro-8-hydroxy-dipropylaminotetralin hydrochloride)(sc-253462, Santa Cruz Biotechnology), was made in 95% Ethanol (B27-B2B, Chemical Control Centre, University of Windsor, Windsor, ON, Canada). Once the stock wsa prepared, 100µL aliquots of the stock solution were stored in disposable scintillation vials at -

20ºC. Prior to recording, one of the 1mM aliquots was diluted to 1µM s(-)-uh-301 in chilled Ringer's solution by serial dilutions.

2.7 Experiment 2: The effect of 5-HT1a receptor antagonists picospritzed onto the olfactory nerve on bulbar odour responses.

The taurocholic acid, amino acid mixture and pheromones were delivered to the olfactory epithelium using the same method previously described (see Section 2.2.5). However, the odour delivery time was reduced to 2 seconds from 5 seconds during experiment 1. This decrease in the odour delivery time still allowed the recording of odour responses, but provided a faster recovery with the smaller amount of odour.

The modulation tests of 5-HT1a antagonists and vehicle controls (Ethanol (1%) and DMSO (1%)) were locally ejected by applying positive pressure pulses ejection from a picospritzer (PICOSPRITZER II, Parker Hannifin Corporation, General Valve Division, Milton, ON, Canada) into the medial portion of the olfactory nerve (Figure 2.5) with a pressure of approximately 10 psi. Glass micropipettes that had a 2-5 µm tip diameter, were filled with the different drug solutions and slowly inserted into the surface of the olfactory nerve to avoid leakage into other areas of the preparation. Once the antagonists and vehicle controls were picospritzed into the olfactory nerve, they diffused to the olfactory bulb. The drugs were picospritzed into the olfactory nerve in order to stimulate 5-HT fibers that ran parallel along the olfactory nerve and into the olfactory bulb. In order to monitor the location of the picospritzed drug, the dye Fast Blue (5%) was added to the solution in the ejection pipette for every experiment. Each trial of the picospritzed modulation test on olfactory responses included OB odour responses under normal conditions, during the application of the picospritzed drugs and after a washout of the drug. There were 3 odour deliveries before any drugs were applied to the preparation; then 3 odour deliveries once the drug had been picospritzed into the olfactory nerve

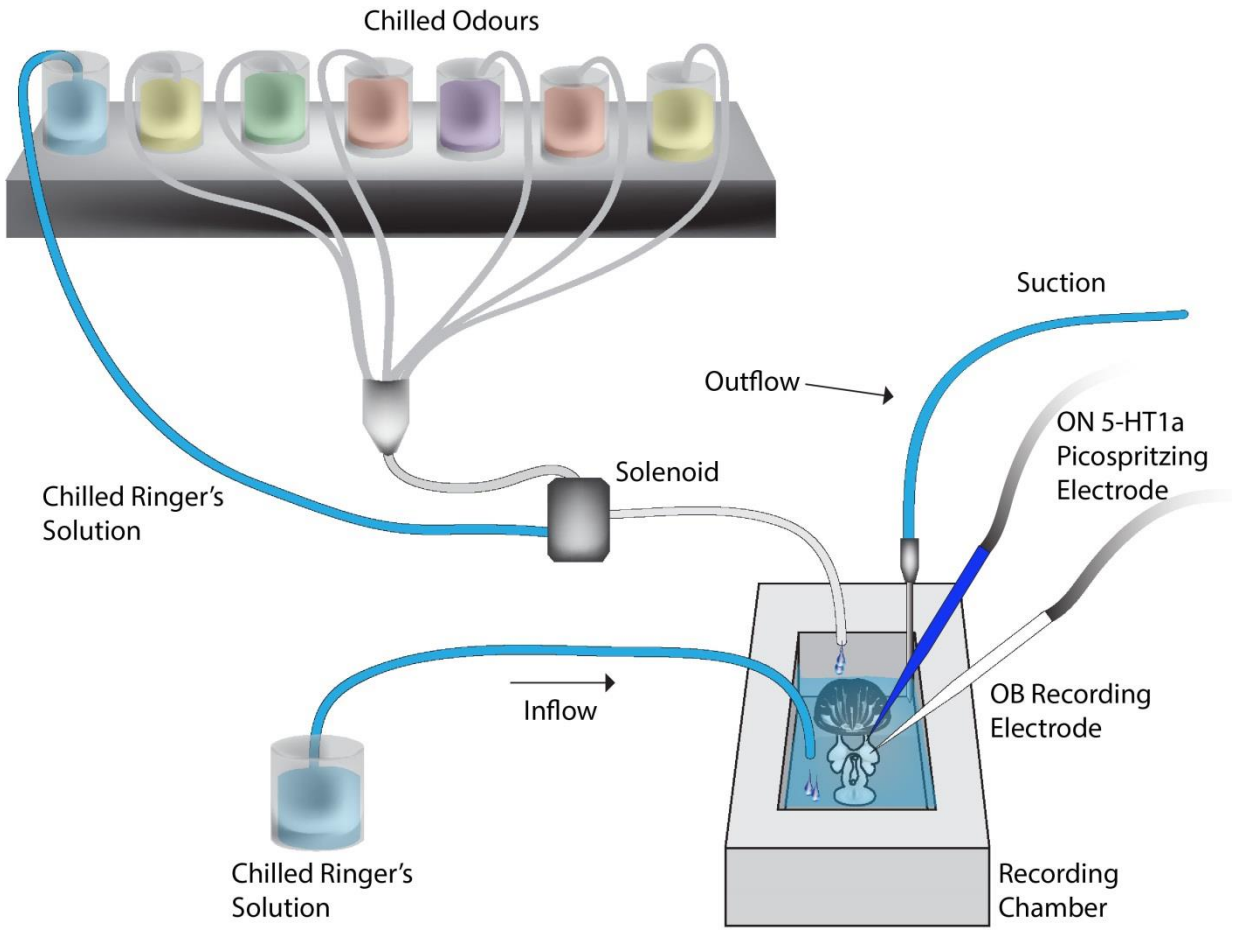
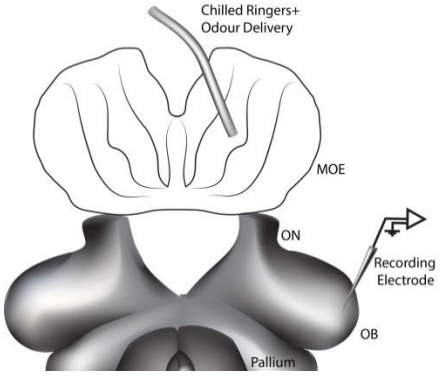
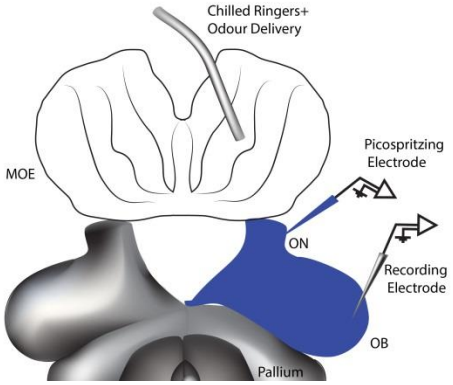


Figure 2.5. An illustration of the 5-HT1a picospritzing setup for experiment 2. The custom-made odour delivery system delivered odours and a constant drip of Ringer's solution to the olfactory epithelium via a 3-way solenoid valve. The solenoid allowed for switching from the background Ringer's solution to an odour. The olfactory-brain preparation in the recording chamber was submerged in chilled Ringer's solution. While an odour was applied to the olfactory epithelium, LFP's were recorded from the olfactory bulb (OB). To test 5-HT modulation of odour responses, a picospritzing electrode was used to inject 5-HT1a antagonists into the olfactory nerve (ON). Illustration modified from Green 2012.

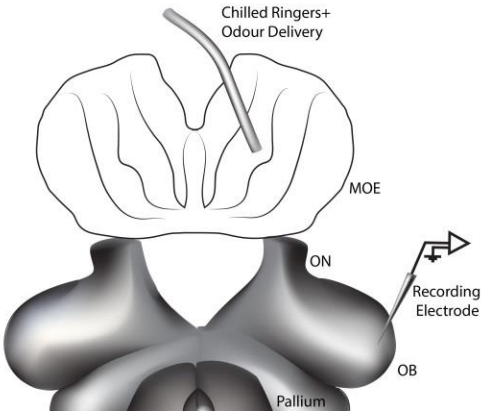
(A) Control (Odour)



(B) Odour + Drug (30 second delivery)



(C) Washout Period (15 minutes)



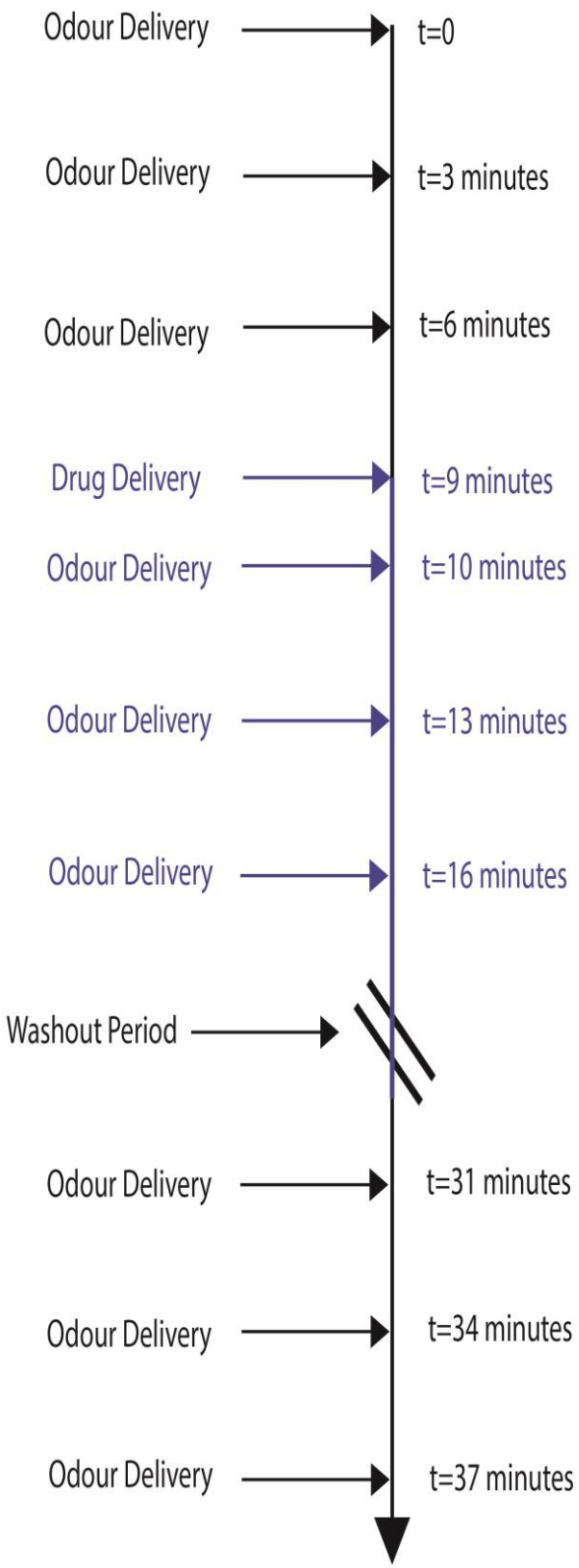


Figure 2.6. The time course for delivery of odourant test solutions in experiment 2. A: An odour was applied to the peripheral olfactory organ between 3 times, with 3 minutes between deliveries, while recording LFP's from the dorsal or lateral region of the olfactory bulb (OB). B: While recording from the OB, a picospritzing electrode was used to inject 5-HT1a antagonists, 5-HT or vehicle blanks into the olfactory nerve (ON) for approximately 30 seconds. A blue solution (5% Fast Blue) was utilized to visualize the picospritzed solution over the ON and OB. The first odour application was delivered within 30 seconds of the completion of picospritzing. The same odour was delivered 2 additional times with 3 minutes between each application. C: A 15 minute washout period was given for the drug to be removed from the system, then the same odour was applied 3 times, with 3 minutes between deliveries. A-C: For perfusion over the olfactory epithelium during odour tests, the 3-way solenoid valve enabled Ringer's solution to be applied for 3 minutes, followed by the odour for 2 seconds, then back to background Ringers solution.

for 30 seconds. These started immediately after the 30 second picospritzing period was complete, then 2 additional odour deliveries were applied 3 minutes apart. Once the odour deliveries in the presence of the drug were complete, a 15 minute washout period was given for recovery from the drug (Grillner et al., 2000). The odour tests following the washout period included 3 odour deliveries after the drug had been removed (Figure 2.6). The vehicle controls comprised of picospritzing 1% DMSO for spiperone and 1% ethanol for s(-)-uh-301. These were picospritzed into the olfactory nerve, followed by OB recordings of odour responses.

2.8 Local field potential recordings

LFP responses were recorded from the dorsal and lateral olfactory bulb in response to odourant test solutions during the 5-HT bath experimentation and the 5-HT picospritzing experimentation (Table 3.1). These odours consisted of: the bile acid acid mixture of L-arginine and L-histidine and a mixture of migratory and reproductive pheromones that consisted of: 3-keto petromyzonol sulfate, 3-keto allocholic acid, petromyzonol sulfate, petromyzonamine disulfate and petromyzosterol disulfate. These odours were previously shown to be detected by the olfactory system of the sea lamprey (Li et al., 1995; Sorensen and Li 1997; Bjerselius et al., 2000; Sorensen 2005). All odours and 5-HT (5-hydroxytryptamine) were dissolved in lamprey Ringer's solution. Ringer's solution did not elicit an olfactory response when tested by itself. In addition, a 1mM solution of 5-HT did not cause an olfactory response when it was applied to the olfactory epithelium by itself (Figure 2.7). This finding confirmed that any 5-HT effects seen were not from 5-HT functioning as an odour. Two 5-HT1a receptor antagonists were used in an attempt to hinder the effect of 5-HT modulation on olfactory responses in the olfactory bulb. Those antagonists were spiperone hydrochloride and s(-)-uh-301 (S(-)-5-fluoro-8-hydroxy-dipropylaminotetralin hydrochloride). Spiperone hydrochloride was dissolved in DMSO and s(-)-

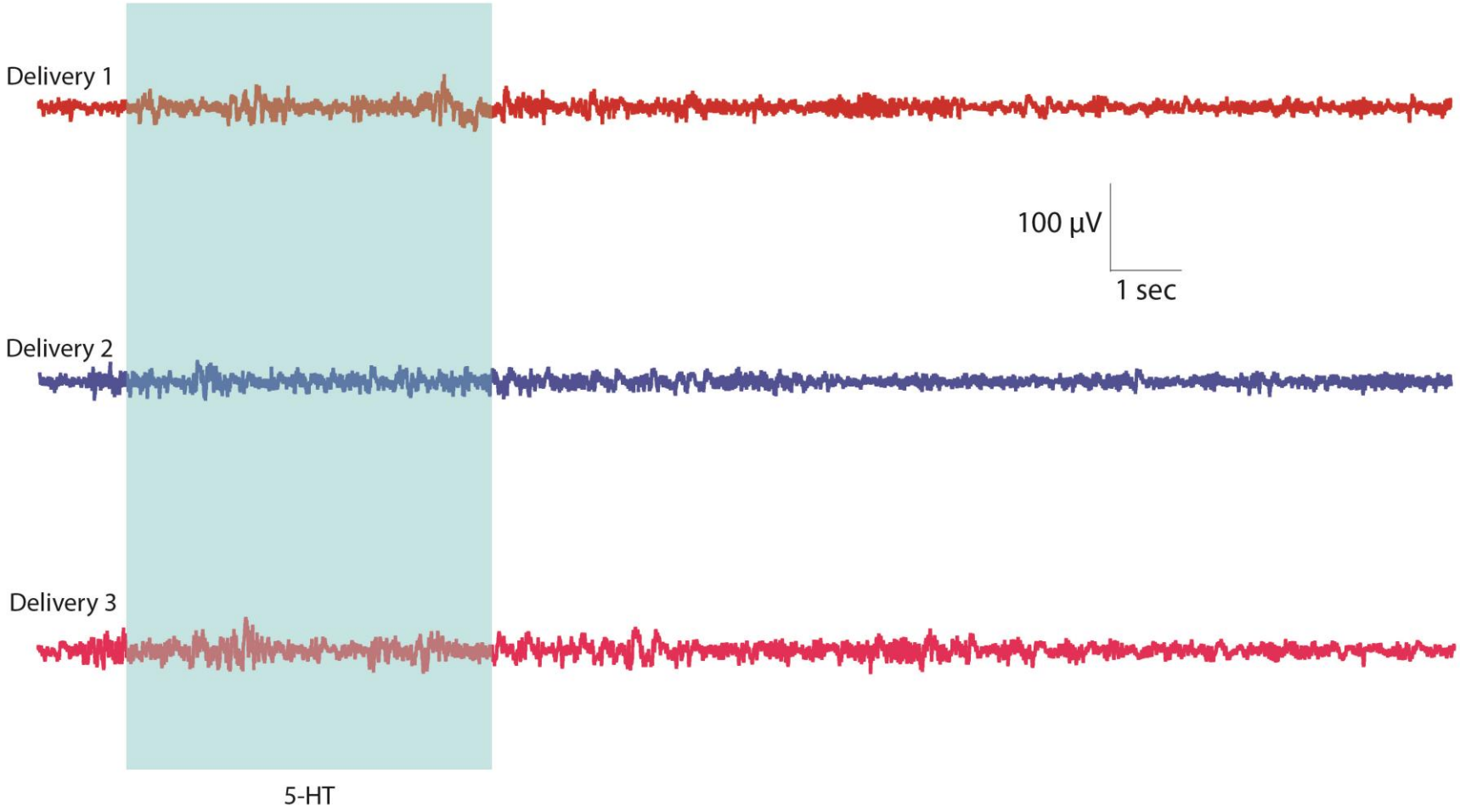


Figure 2.7. An example of LFP responses in the dorsal olfactory bulb to an application of 1mM 5-HT. The 5-HT was delivered 3 times and no olfactory responses were elicited in any of the deliveries. The 5-HT was applied for 5 seconds (blue shaded box) to the olfactory epithelium and each trace lasts for 20 seconds.

uh-301 was dissolved in ethanol. Neither DMSO, nor ethanol had an effect on modulating odour responses when tested by in the same manner as spiperone and s(-)-uh-301. In order to determine if 5-HT had a modulatory effect on olfactory responses in the olfactory bulb and if 5-HT1a antagonists would block the effect of 5-HT, three characteristics were analyzed: the length of the odour response, the number of peaks in a response and the average peak amplitude of a response. In order to be included in the data analysis, a minimum of three consistent odour responses were required.

2.9 Data analysis

Each preparation, which included a recording from the dorsal or lateral region of the olfactory bulb or both, was investigated for the effect of 5-HT and of 5-HT1a antagonists on different odour responses. A recording from an animal was included in the analysis if there was no response to the blanks (Ringers, DMSO or Ethanol) and consistent responses were seen across 3 deliveries of one odour. Once experimentation was complete, the recording was digitally filtered through a low pass filter with the cut off frequency at 100 Hz to ensure good signal to noise ratio using LabChart software (version 6.1.3, ADInstruments) and analyzed for spatial and temporal characteristics. For every odour application, the mean and standard deviation of the baseline was calculated for a 5 second interval before the application. This allowed for the responses to be clearly distinguishable from the baseline and to determine the maximum peak amplitude. The odour response threshold was calculated as 3-times the baseline standard deviation. Odour-evoked LFP's within an odour response that were higher than the response threshold were counted as part of the response. The threshold was used to distinguish odour-evoked LFP responses from background spontaneous neuron activity and baseline deviations. LFP's were also separated from spontaneous neuron activity by comparing the odour

responses to the rates of the spontaneous activity and confirming that the odour-evoked responses were consistent and repeatable across multiple deliveries. If a response did not meet those requirements, then it was not included in the analysis. The number of peaks, peak amplitude, time of the peak, interpeak amplitude and the duration of the response were calculated within every LFP response. The peak amplitude was calculated as the change in voltage between the pre peak baseline mean and the peak maximum. In order to further analyze each peak, it was highlighted and run through a program called R (Version 2.15.3, R Development Core Team 2012) using custom written routines. This allowed for the time at which 10%, 50% and 90% pre and post peak maximum amplitude to be determined. From this, the duration of the response was calculated as the time between 10% pre maximum amplitude of the first peak of the response and 10% post maximum amplitude of the last peak in the response (Figure 2.8).

2.10 Experiment 1: Determination of inhibitory and excitatory effects

5-HT was determined to have an inhibitory effect on odour-evoked LFP's when the average peak amplitude during the period that 5-HT was in the recording chamber was lower than the average peak amplitude before 5-HT was applied to the chamber, and returned to control values during the washout period. The recordings that showed excitatory effects had a higher average peak amplitude when 5-HT filled the chamber than before 5-HT was applied to the chamber, and an inhibitory effect when the average peak amplitude was lower.

The extent of inhibition and excitation that the 5-HT bath application caused on the peak amplitude in every animal was calculated. For each animal, the average peak amplitude was calculated for odour responses during 5-HT bath application and for odour responses that occurred before 5-HT was applied. The value calculated for the average peak amplitude during 5-HT was divided by the value for the average peak amplitude before 5-HT. If the resulting

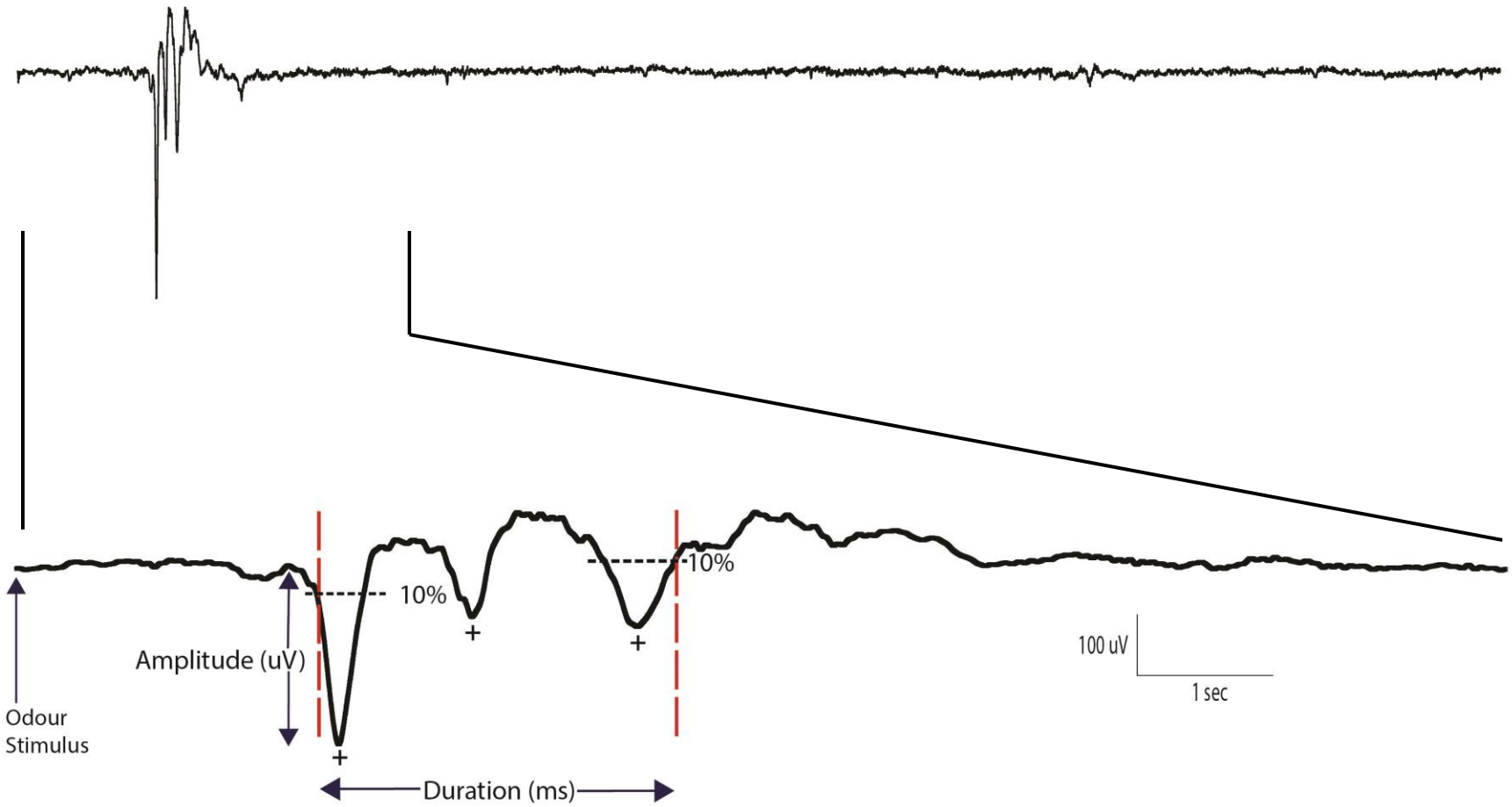


Figure 2.8. An example of a local field potential response (LFP) recorded from the olfactory bulb to an odourant test solution applied to the olfactory epithelium. Three characteristics were measured in every LFP response: number of peaks in a response (denoted by a +), the amplitude of each peak in a response (μV) and the duration of the response (ms). The number of peaks in a response was calculated by counting the total number of peaks in a response. The peak amplitude was measured as the voltage change between the pre-peak baseline and the peak maximum. The response duration was calculated as the time between the value at 10% pre maximum amplitude of the first peak in the response and the value at 10% post maximum amplitude of the last peak in the response. The top trace is 20 seconds in length and the bottom trace is a 5 second zoomed in view of the top trace.

value was less than 1, then it was considered inhibitory. If the ratio was greater than 1, then it would be excitatory. These values were calculated for every odour in each animal in the dorsal and lateral OB. 5-HT was determined to have an inhibitory effect on response duration when the average length of the odour-evoked LFP responses were shorter during 5-HT bath application than the average length of responses before 5-HT was applied to the chamber. The recordings that showed excitatory effects had a longer average response duration when 5-HT was in the chamber than before 5-HT was applied to the chamber.

In order to determine the modulatory effect that 5-HT had on response duration, the average response duration was calculated for odour responses that occurred during the 5-HT bath application and divided by the average response duration for odour responses that occurred before 5-HT was applied to the chamber. If the resulting value was less than 1, then it was considered inhibitory. If the value was greater than 1, then it would be excitatory. These values were calculated for every odour in each animal in the dorsal and lateral OB.

5-HT was determined to have an inhibitory effect on the number of peaks when the average number of peaks in an odour-evoked LFP response was lower during 5-HT bath application than the average number of peaks before 5-HT was applied to the chamber. The recordings that showed excitatory effects had a higher number of peaks when 5-HT was in the chamber than before 5-HT was applied to the chamber.

In order to determine the modulatory effect that 5-HT had on the number of peaks in a response, the average number of peaks was calculated for odour responses that occurred during the 5-HT bath application and divided by the average number of peaks for odour responses that occurred before 5-HT was applied to the chamber If the resulting value was less than 1, then it

was considered inhibitory. If the value was greater than 1, then it would be excitatory. These values were calculated for every odour in each animal in the dorsal and lateral OB**.**

2.11 Experiment 2: Determination of inhibitory and excitatory effects

The extent of excitation that spiperone hydrochloride and s(-)-uh-301 caused on the peak amplitude in every animal was calculated. For each animal, the average peak amplitude was calculated for odour responses when the 5-HT1a antagonists were in the OB and for odour responses that occurred before they were applied. The value calculated for the average peak amplitude when spiperone and s(-)-uh-301 were in the OB was divided by the value for the average peak amplitude before the antagonists were applied. If the resulting ratio was less than 1, then it was considered inhibitory. If the value was greater than 1, then it was excitatory. These values were calculated for every odour in each animal in the dorsal and lateral OB for both 5-HT1a antagonists.

Spiperone and s(-)-uh-301 were determined to have an inhibitory effect on response duration when the average length of the odour-evoked LFP responses were shorter when the antagonists were in the OB than the average length of responses before the antagonists were applied. The recordings that showed excitatory effects had a longer average response duration when the antagonists were in the OB than before they were applied.

In order to determine the modulatory effect that the 5-HT1a antagonists had on response duration, the average response duration was calculated for odour responses that occurred during the time that the antagonists were in the OB and divided by the average response duration for odour responses that occurred before the antagonists were applied to the OB. If the resulting value was less than 1, then it was considered inhibitory. If the value was greater than 1, then it was excitatory. These values were calculated for every odour in each animal in the dorsal and

lateral OB for both 5-HT1a antagonists.

Spiperone and s(-)-uh-301 were determined to have an inhibitory effect on the number of peaks when the average the number of peaks in the odour-evoked LFP responses were shorter when the antagonists were in the OB than the number of peaks in the response before the antagonists were applied. The recordings that showed excitatory effects had a larger number of peaks when the antagonists were in the OB than before they were applied.

In order to determine the modulatory effect that the 5-HT1a antagonists had on the number of peaks, the average number of peaks was calculated for odour responses that occurred during the time that the antagonists were in the OB and divided by the average number of peaks for odour responses that occurred before the antagonists were applied to the OB. If the resulting value was less than 1, then it was considered inhibitory. If the value was greater than 1, then it would be excitatory. These values were calculated for every odour in each animal in the dorsal and lateral OB for both 5-HT1a antagonists.

Chapter 3

Results

Table 3.1. The number of animals tested for Experiment 1 (A) and Experiment 2 (B). The odours were: 0.1mM taurocholic acid (TCA), a 0.001mM mixture of sex and migratory pheromones (3KACA, 3KPZS, PZS, PADS, PSDS) and a 0.1mM amino acid mixture (AA) of L-arginine and L-histidine for bath experiments and 1mM AA for picospritzing experiments. Recordings in both experiments were taken from the dorsal and lateral regions of the OB to examine 5-HT spatial differences. N tested denotes the number of animals tested for a given odour, N responding denotes the number of animals that responded to each odour and % responding represents the percentage of animals that responded to each odour.

A)

Experiment 1: 5-HT bath application				
OB Region	**Odour**	**N tested**	**N responding to odour**	**% responding to odour**
Dorsal	TCA	12	12	100
	AA	8	4	50
	Pheromones	11	9	83.3
Lateral	TCA	4	0	0
	AA	8	8	100
	Pheromones	4	0	0

B)

Experiment 2: Picospritzing of 5-HT1a antagonists				
OB Region	**Odour**	**N tested**	**N responding to odour**	**% responding to odour**
Dorsal	TCA	15	15	100
	Arg/His	5	0	0
	Pheromones	11	10	90.9
Lateral	TCA	2	0	0
	Arg/His	9	9	100
	Pheromones	2	0	0

3.1 Experiment 1: 5-HT bath application effects on the peak amplitude of odour-evoked LFP responses

During the 5-HT bath tests, the average peak amplitude of odour-evoked LFP responses changed during the 5-HT treatment and returned to control values 80% of the time during the washout period (Appendix Table A1-A6).

In the dorsal OB region, 5 out of 12 animals showed a decrease in peak amplitude (Figure 3.1), while 4 out of 12 showed an increase (Figure 3.2) and 3 showed no effect during 5-HT bath treatment while testing the odour, 0.1mM taurocholic acid. When examining LFP responses to the 0.001mM pheromone mixture in the dorsal OB, the average peak amplitude was attenuated in 7 out of 11 animals (Figure 3.3) during the 1mM 5-HT bath application, 3 out of 11 were enhanced (Figure 3.4) and 1 showed no effect. LFP responses to the 0.1mM amino acid mixture in the dorsal OB caused the average peak amplitude to be attenuated in 3 out of 4 animals (Figure 3.5) while causing enhancing effects in 1 animal (Figure 3.6). Similar results were seen on peak amplitude in the lateral OB. The 5-HT bath caused the average peak amplitude of the amino acid mixture in the lateral OB to be attenuated in 6 out of the 8 animals (Figure 3.7) and enhanced in 2 out of the 8 animals (Figure 3.8).

Based on these results, the effect of 5-HT modulation on peak amplitude of pheromones and taurocholic acid by 5-HT bath application is unclear, but an attenuating effect is seen on peak amplitude of amino acid responses in both regions of the OB during most tests (Figure 3.9).

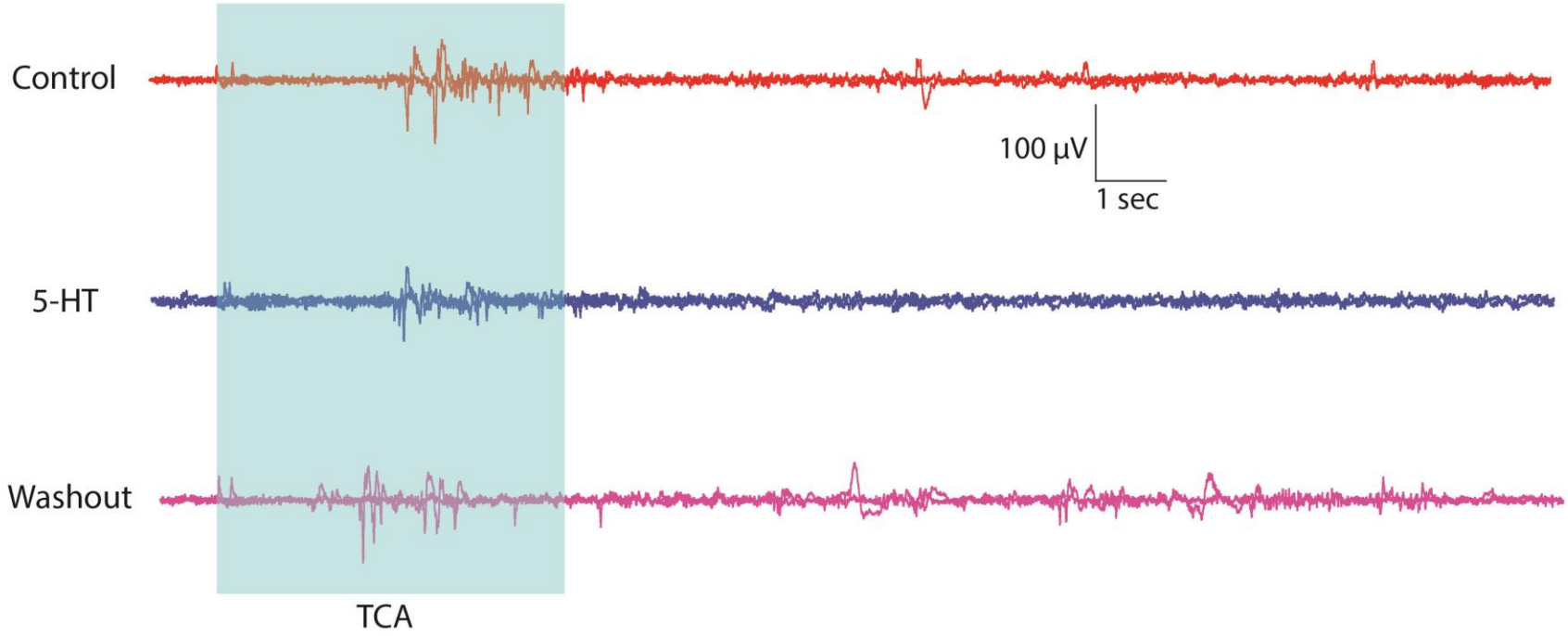


Figure 3.1. Experiment 1: An example of LFP responses in the dorsal OB to 0.1mM TCA showing an inhibitory effect on peak amplitude. During the control period (3 superimposed red traces), TCA was applied as an odour test while Ringer's solution was perfused through the recording chamber. The 3 superimposed blue traces represent LFP responses to TCA when the recording chamber contained 1mM 5-HT. The 3 superimposed pink traces show LFP activity when TCA was applied during the washout period when 5-HT was displaced from the system by Ringer's solution. TCA was applied for 5 seconds (blue shaded box) to the olfactory epithelium and each trace lasted for 20 seconds.

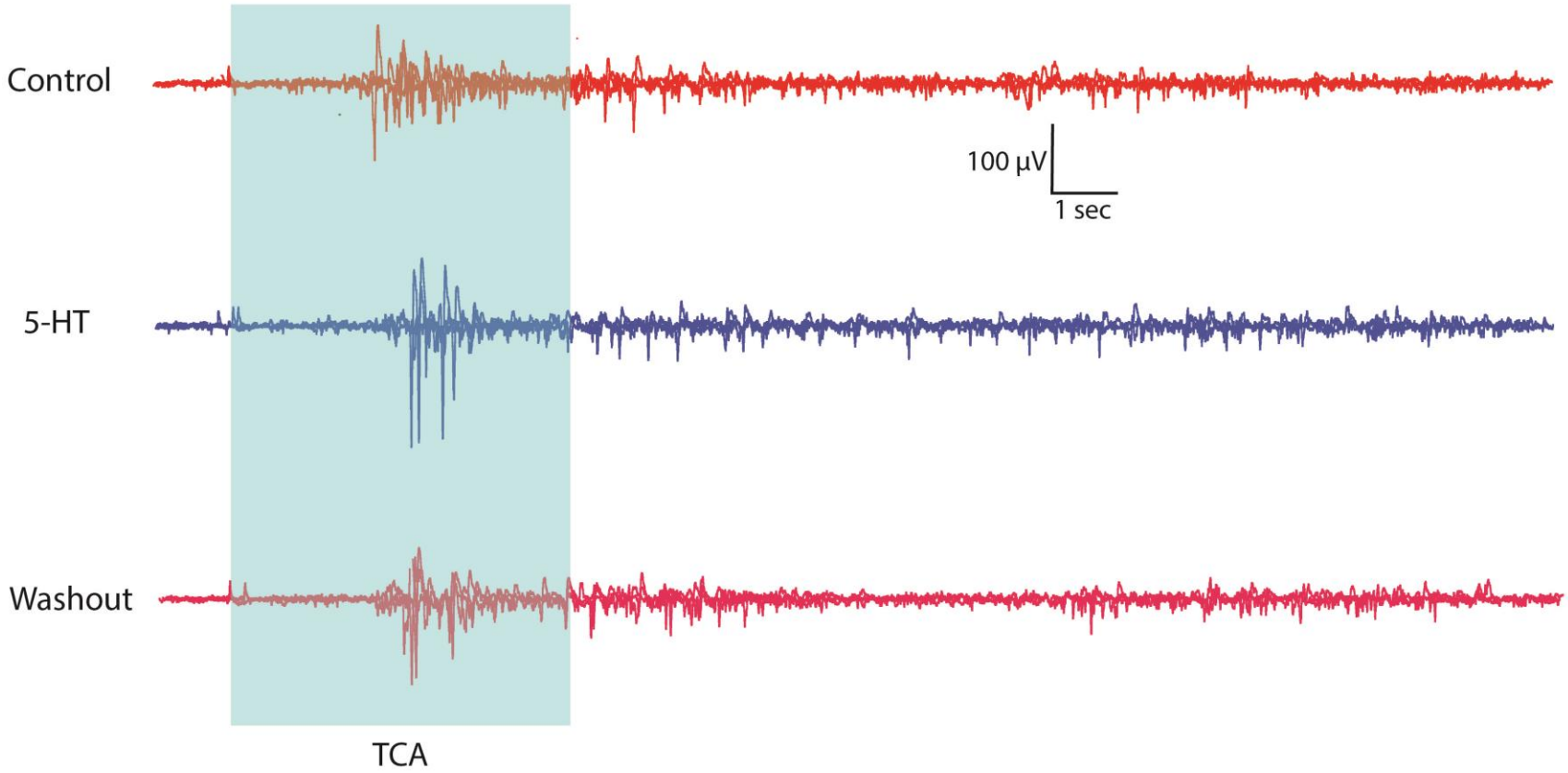


Figure 3.2. Experiment 1: An example of LFP responses in the dorsal OB to 0.1mM TCA showing an excitatory effect on peak amplitude. During the control period (3 superimposed red traces), TCA was applied as an odour test while Ringer's solution was perfused through the recording chamber. The 3 superimposed blue traces represent LFP responses to TCA when the recording chamber contained 1mM 5-HT. The 3 superimposed pink traces show LFP activity when TCA was applied during the washout period when 5-HT was displaced from the system by Ringer's solution. TCA was applied for 5 seconds (blue shaded box) to the olfactory epithelium and each trace lasted for 20 seconds.

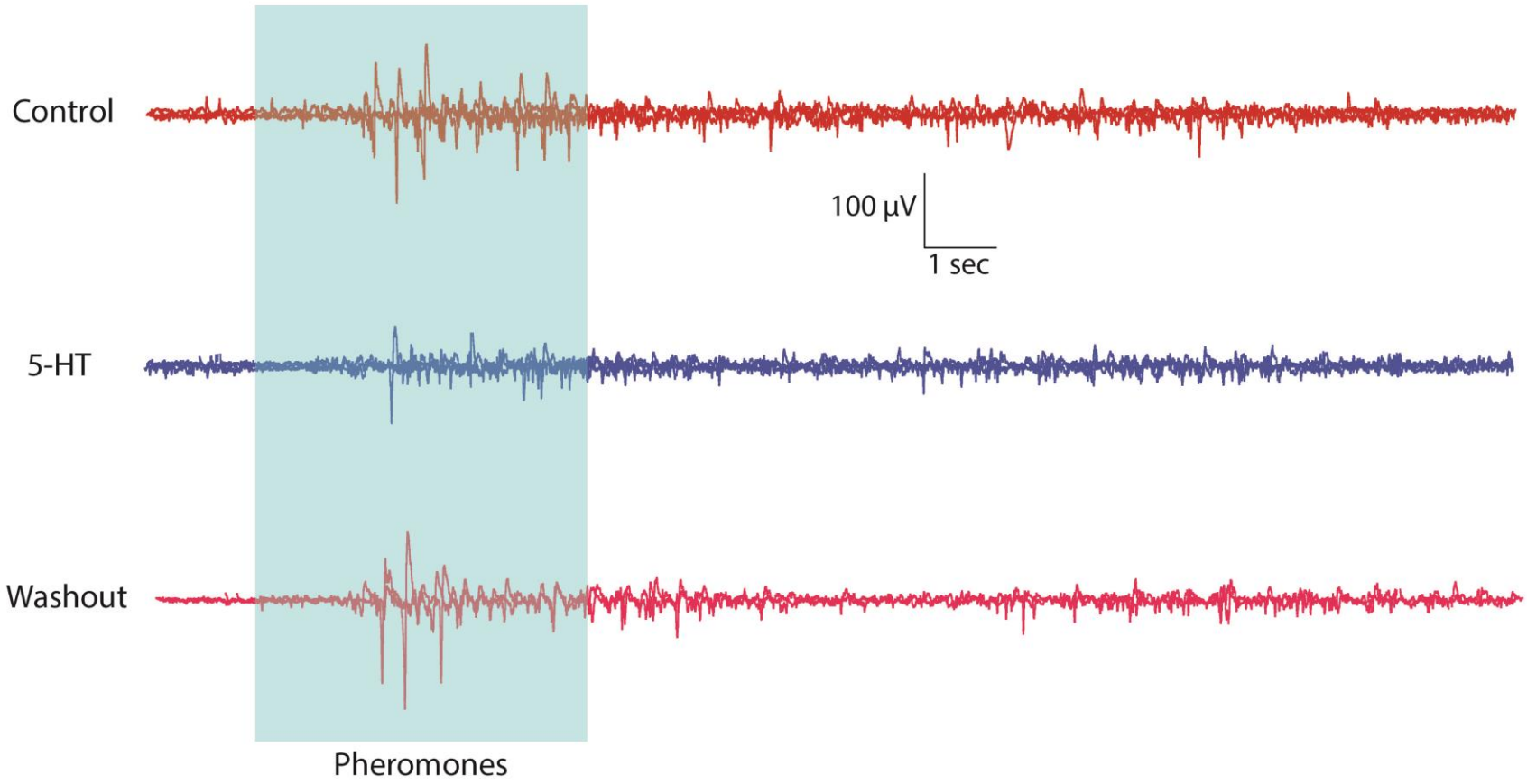


Figure 3.3. Experiment 1: An example of LFP responses in the dorsal OB to a 0.001mM pheromone mixture showing an inhibitory effect on peak amplitude. During the control period (3 superimposed red traces), the pheromone mixture was applied as an odour test while Ringer's solution was perfused through the recording chamber. The 3 superimposed blue traces represent LFP responses to the pheromones when the recording chamber contained 1mM 5-HT. The 3 superimposed pink traces show LFP activity when the pheromone mixture was applied during the washout period when 5-HT was displaced from the system by Ringer's solution. The pheromones were applied for 5 seconds (blue shaded box) to the olfactory epithelium and each trace lasted for 20 seconds.

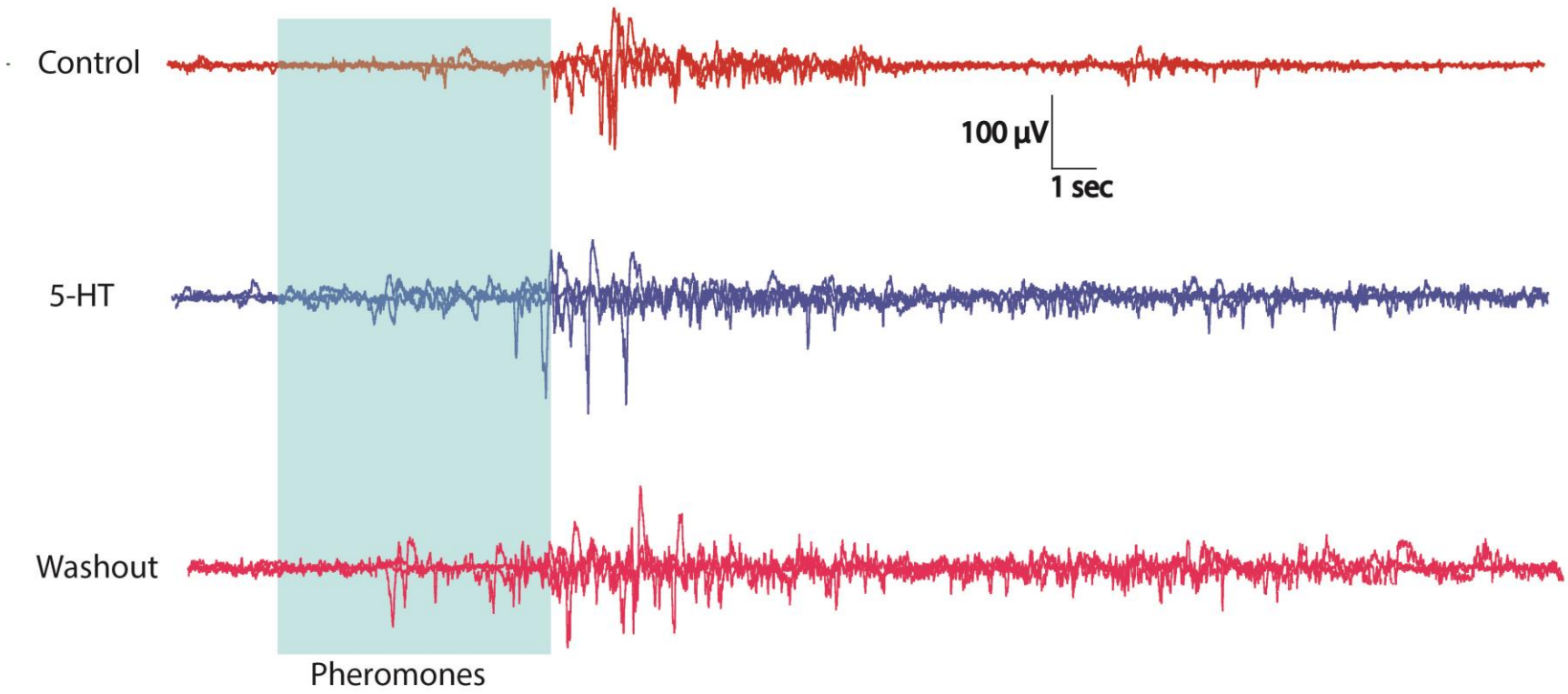


Figure 3.4. Experiment 1: An example of LFP responses in the dorsal OB to a 0.001mM pheromone mixture showing an excitatory effect on peak amplitude. During the control period (3 superimposed red traces), the pheromone mixture was applied as an odour test while Ringer's solution was perfused through the recording chamber. The 3 superimposed blue traces represent LFP responses to the pheromones when the recording chamber contained 1mM 5-HT. The 3 superimposed pink traces show LFP activity when the pheromone mixture was applied during the washout period when 5-HT was displaced from the system by Ringer's solution. The pheromones were applied for 5 seconds (blue shaded box) to the olfactory epithelium and each trace lasted for 20 seconds.

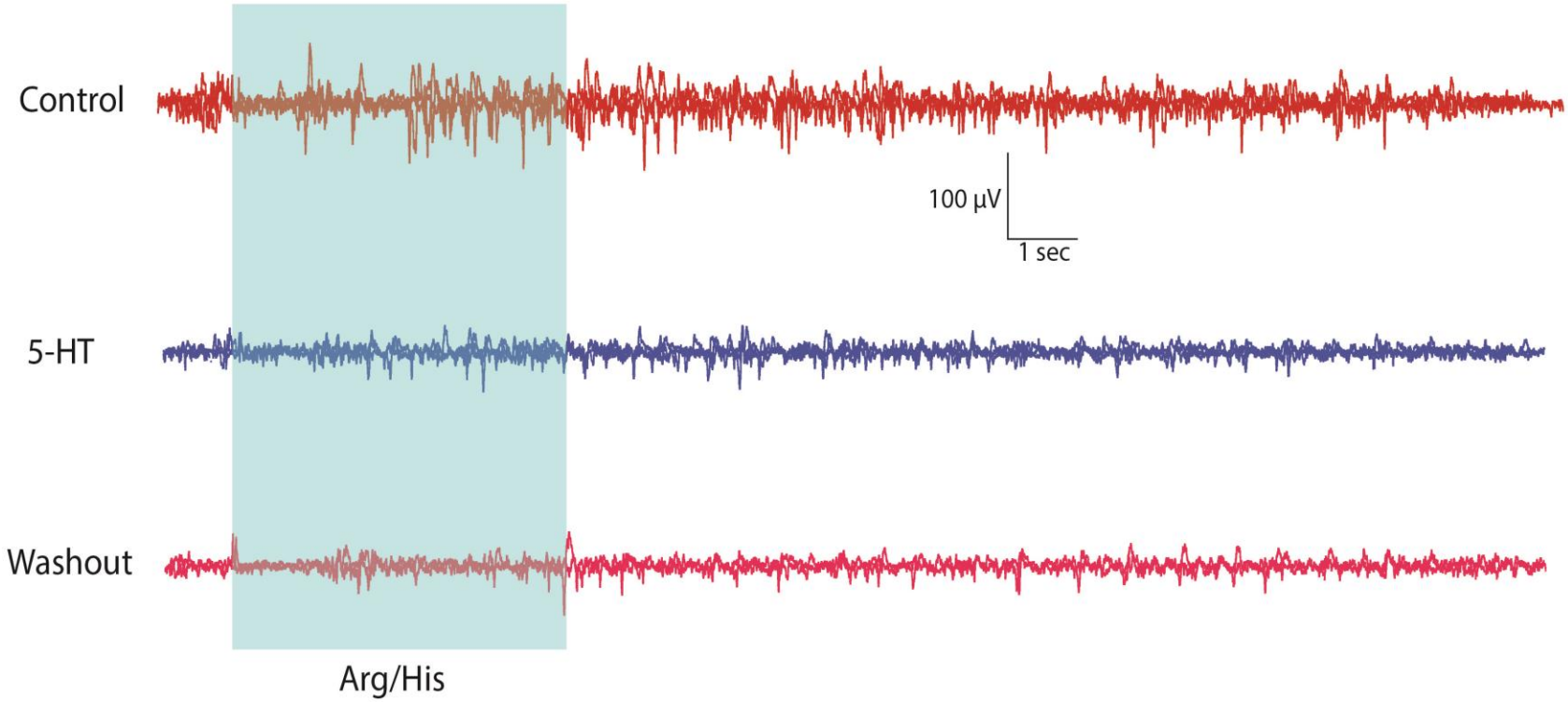


Figure 3.5. Experiment 1: An example of LFP responses in the dorsal OB to a 0.1mM amino acid mixture showing an inhibitory effect on peak amplitude. During the control period (3 superimposed red traces), the amino acid mixture was applied as an odour test while Ringer's solution was perfused through the recording chamber. The 3 superimposed blue traces represent LFP responses to the amino acids when the recording chamber contained 1mM 5-HT. The 3 superimposed pink traces show LFP activity when the amino acid mixture was applied during the washout period when 5-HT was displaced from the system by Ringer's solution. The amino acids were applied for 5 seconds (blue shaded box) to the olfactory epithelium and each trace lasted for 20 seconds.

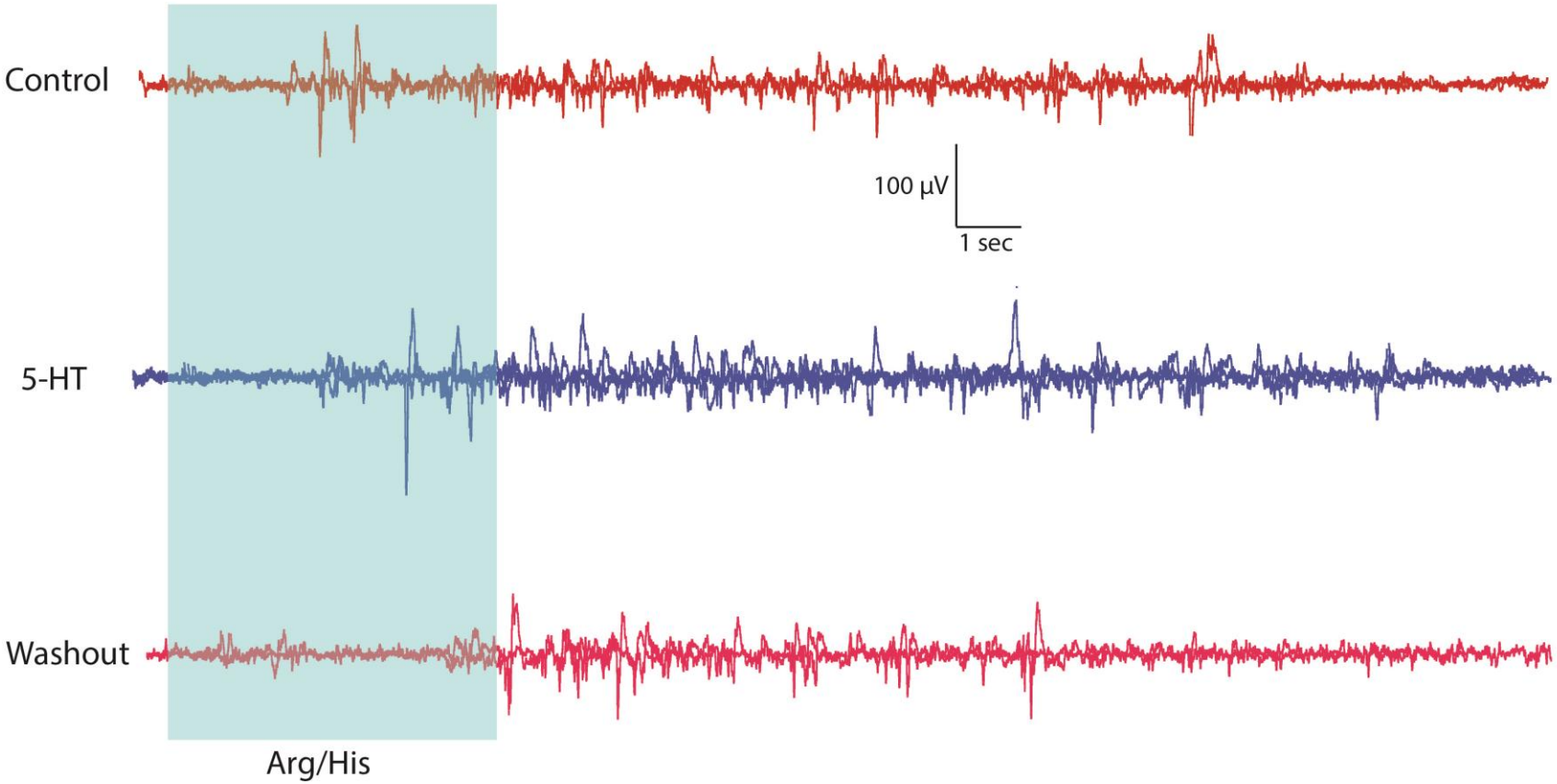


Figure 3.6. Experiment 1: An example of LFP responses in the dorsal OB to a 0.1mM amino acid mixture showing an excitatory effect on peak amplitude. During the control period (3 superimposed red traces), the amino acid mixture was applied as an odour test while Ringer's solution was perfused through the recording chamber. The 3 superimposed blue traces represent LFP responses to the amino acids when the recording chamber contained 1mM 5-HT. The 3 superimposed pink traces show LFP activity when the amino acid mixture was applied during the washout period when 5-HT was displaced from the system by Ringer's solution. The amino acids were applied for 5 seconds (blue shaded box) to the olfactory epithelium and each trace lasted for 20 seconds.

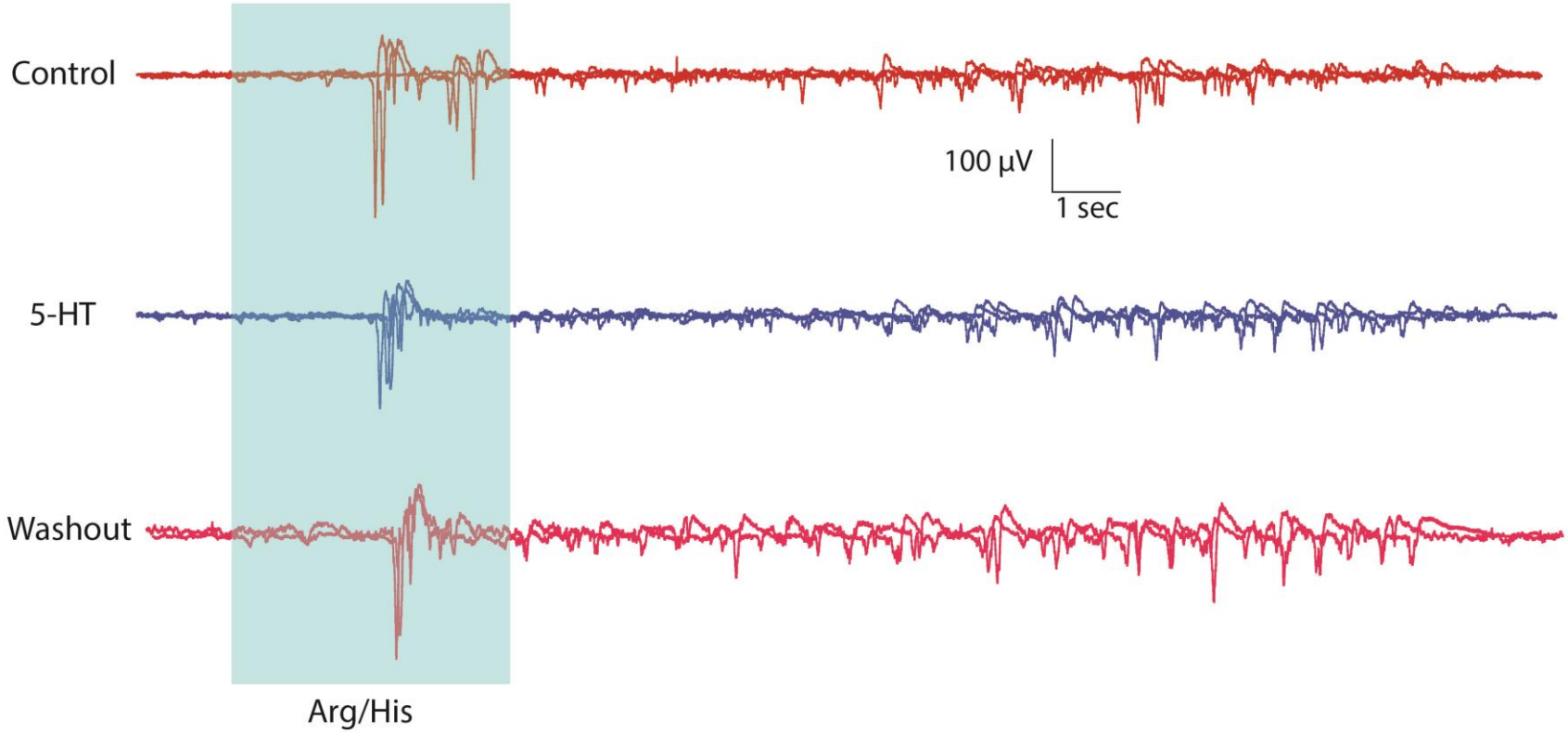


Figure 3.7. Experiment 1: An example of LFP responses in the lateral OB to a 0.1mM amino acid mixture showing an inhibitory effect on peak amplitude. During the control period (3 superimposed red traces), the amino acid mixture was applied as an odour test while Ringer's solution was perfused through the recording chamber. The 3 superimposed blue traces represent LFP responses to the amino acids when the recording chamber contained 1mM 5-HT. The 3 superimposed pink traces show LFP activity when the amino acid mixture was applied during the washout period when 5-HT was displaced from the system by Ringer's solution. The amino acids were applied for 5 seconds (blue shaded box) to the olfactory epithelium and each trace lasted for 20 seconds.

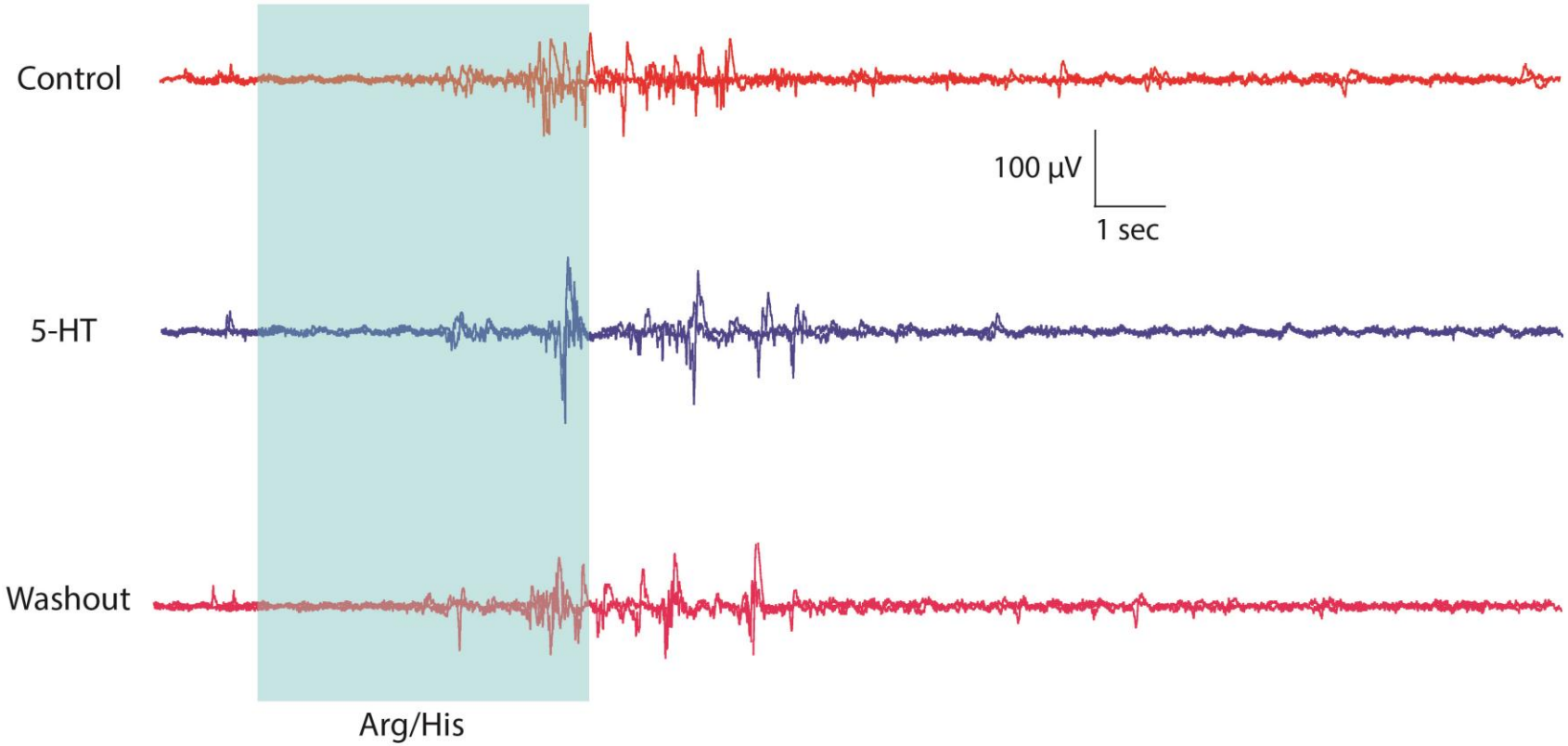


Figure 3.8. Experiment 1: An example of LFP responses in the lateral OB to a 0.1mM amino acid mixture showing an excitatory effect on peak amplitude. During the control period (3 superimposed red traces), the amino acid mixture was applied as an odour test while Ringer's solution was perfused through the recording chamber. The 3 superimposed blue traces represent LFP responses to the amino acids when the recording chamber contained 1mM 5-HT. The 3 superimposed pink traces show LFP activity when the amino acid mixture was applied during the washout period when 5-HT was displaced from the system by Ringer's solution. The amino acids were applied for 5 seconds (blue shaded box) to the olfactory epithelium and each trace lasted for 20 seconds.

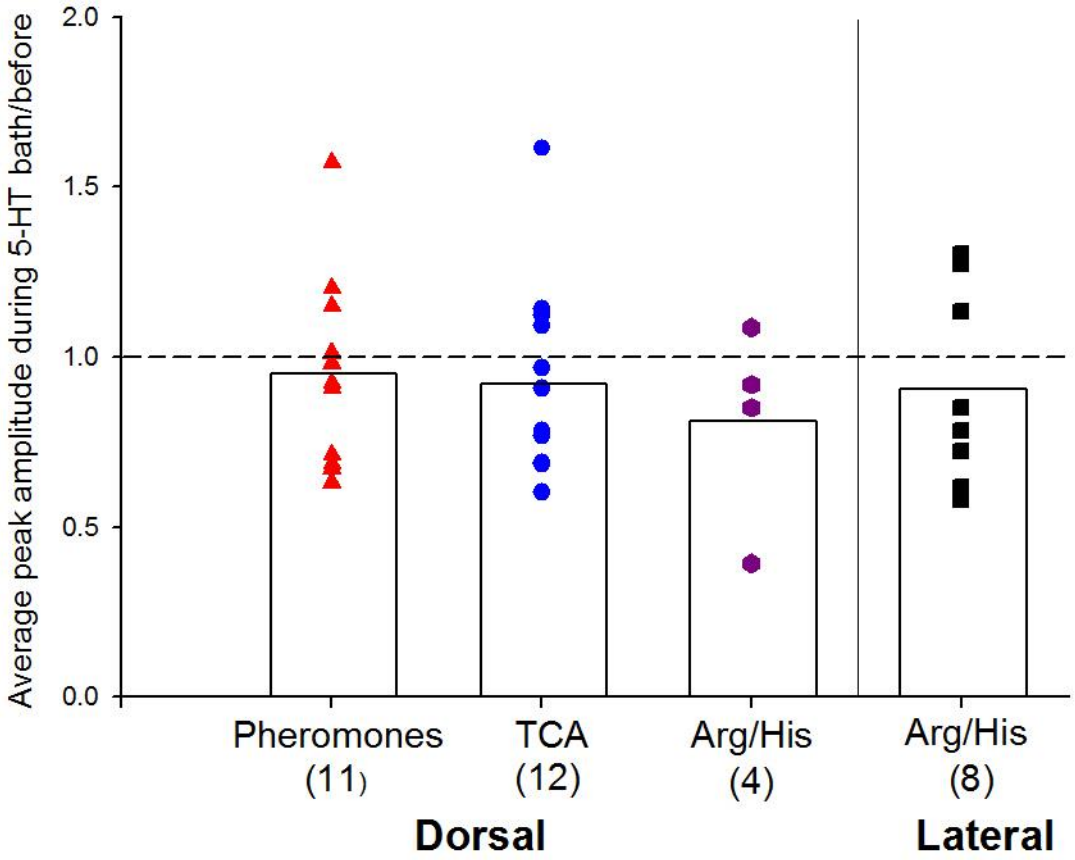


Figure 3.9. Experiment 1: The effect of 1mM 5-HT bath application on the peak amplitude (µV) of LFP odour-evoked responses in the dorsal and lateral olfactory bulb. The x-axis represents the tested odours with the number of animals tested of each in brackets. The solid line separates the two different regions of the OB in which the odours were tested. The y-axis represents the value calculated from the ratio of the average peak amplitude during 5-HT bath application divided by the average peak amplitude before 5-HT bath application. Each point on the graph symbolizes one animal and each odour is shown by a different colour and shape. If a point is below the dashed line (<1), then it is considered inhibitory. If a point is above the dashed line (>1), then it is considered excitatory. The bar graph represents the standard mean of the response magnitude for each odour.

3.2 Experiment 1: 5-HT bath application effects on temporal aspects of odour-evoked LFP responses.

During the 5-HT bath treatment, 5 out of 12 animals displayed a shorter response, 5 out of 12 showed a longer response, while 2 remained unchanged during TCA applications in the dorsal OB (Figure 3.10). When testing the pheromone mixture in the dorsal OB during the 5-HT bath, 7 out of 11 animals displayed shorter responses, 3 animals had longer responses and 1 animal remained unchanged (Figure 3.10). When examining the amino acid mixture in the dorsal OB, all animals showed a shorter response during 5-HT bath (Figure 3.10). In the lateral OB, 2 out of 8 animals showed a shorter response, 5 animals showed a longer response, while 1 showed no change during the 5-HT bath (Figure 3.10).

When investigating the number of peaks, 6 out 11 animals showed a decreased number of peaks, 4 animals showed an increased number of peaks while 1 displayed no change when applying the pheromone mixture to the dorsal OB (Figure 3.11). During TCA applications, 4 out of 12 animals showed a decreased number of peaks, 3 out of 12 displayed an increased number of peaks, while 5 animals showed no change in the dorsal OB (Figure 3.11). Three out of 4 animals showed a decreased number of peaks and 1 showed an increased number of peaks in the dorsal OB when applying amino acids (Figure 3.11). In the lateral OB, 4 animals out of 8 showed a decreased number of peaks, 3 animals showed a greater number of peaks, while 1 displayed no change when testing amino acids during the 5-HT bath (Figure 3.11).

Therefore, 5-HT bath modulates the temporal parameters of amino acids, but the effect of bath applied 5-HT on the temporal parameters of TCA and pheromones is unclear.

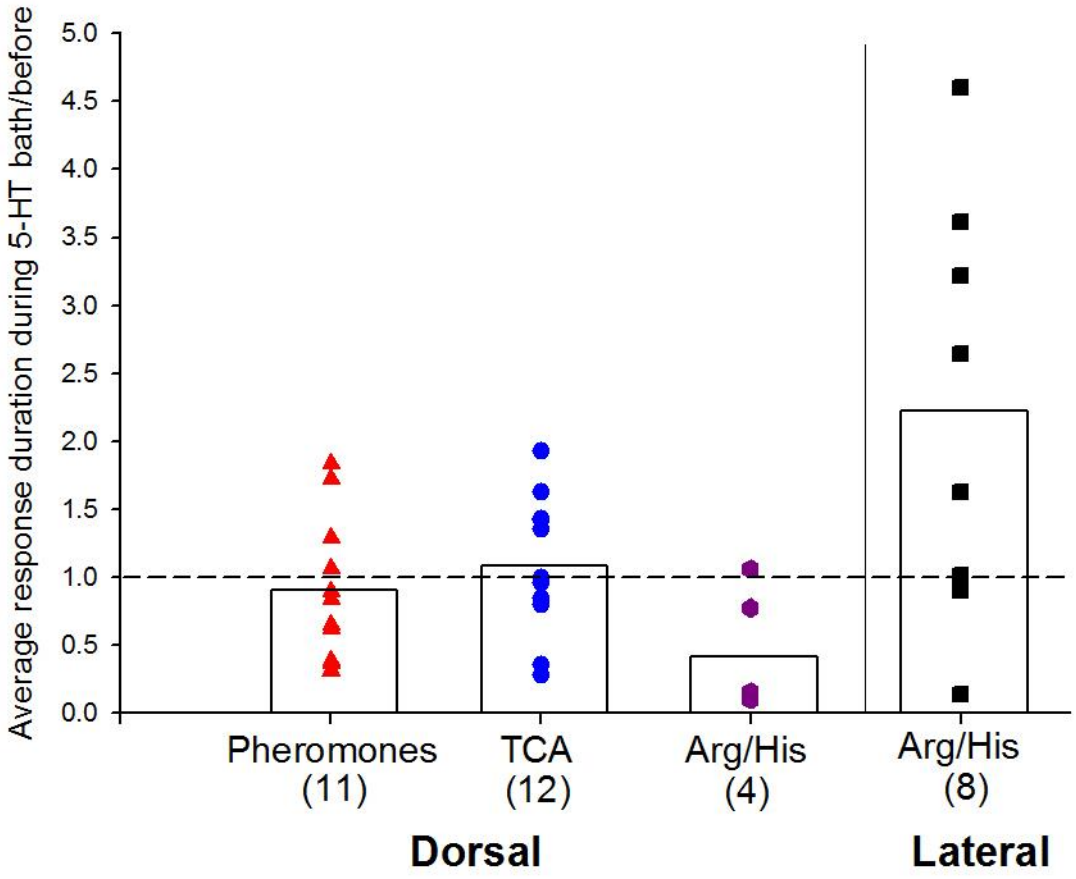


Figure 3.10. Experiment 1: The effect of 1mM 5-HT bath application on the response duration (ms) of LFP odour-evoked responses in the dorsal and lateral olfactory bulb. The x-axis represents the tested odours with the number of animals tested of each in brackets. The solid line separates the two different regions of the OB in which the odours were tested. The y-axis represents the value calculated from the ratio of the average response duration during 5-HT bath application divided by the average response duration before 5-HT bath application. Each point on the graph symbolizes one animal and each odour is shown by a different colour and shape. If a point is below the dashed line (<1), then it is considered inhibitory. If a point is above the dashed line (>1), then it is considered excitatory. The bar graph represents the standard mean of the response magnitude for each odour.

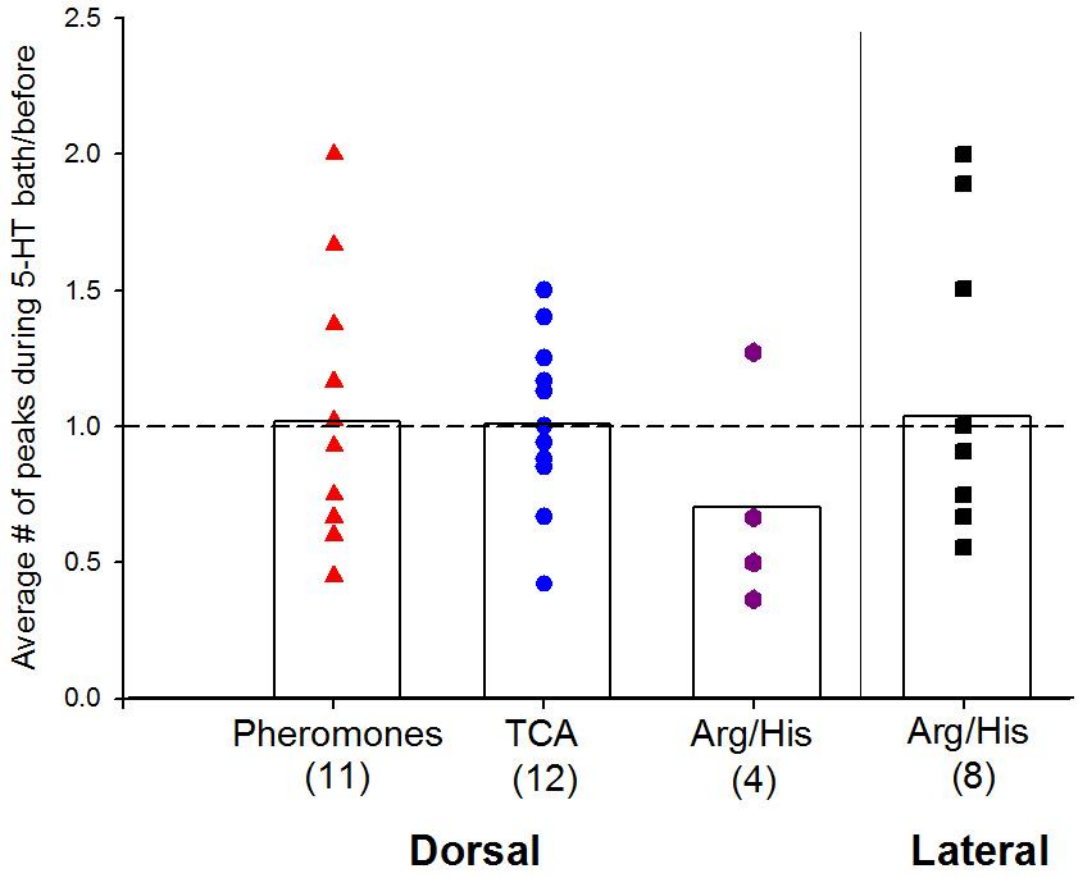


Figure 3.11. Experiment 1: The effect of 1mM 5-HT bath application on the number of peaks in LFP odour-evoked responses in the dorsal and lateral olfactory bulb. The x-axis represents the tested odours with the number of animals tested of each in brackets. The solid line separates the two different regions of the OB in which the odours were tested. The y-axis represents the value calculated from the ratio of the average number of peaks during 5-HT bath application divided by the average number of peaks before 5-HT bath application. Each point on the graph symbolizes one animal and each odour is shown by a different colour and shape. If a point is below the dashed line (<1), then it is considered inhibitory. If a point is above the dashed line (>1), then it is considered excitatory. The bar graph represents the standard mean of the response magnitude for each odour.

3.3 Experiment 2: Effect of picospritzing the 5-HT1a antagonists and 5-HT + antagonist on the average peak amplitude of odour-evoked LFP responses.

The application of 10µM spiperone caused excitatory effects on the average peak amplitude of TCA in 8 out of 11 animals (Figure 3.12) while an attenuating effect was seen in 3 animals. When examining LFP responses to the 0.001mM pheromone mixture in the dorsal OB, the average peak amplitude during spiperone was enhanced in 5 out of 7 animals (Figure 3.13), attenuated in 1 animal and saw no change in 1 animal. The average peak amplitude of amino acid LFP responses was enhanced in 4 out of 6 animals (Figure 3.14), while 1 saw no change and 1 was attenuated in the lateral OB during spiperone. When applied by picospritzer, 1% DMSO, the vehicle for spiperone, did not affect the peak amplitude in the dorsal or lateral OB (Figure 3.15).

The average peak amplitude of LFP responses was enhanced in all animals during application of s(-)-uh-301 when testing 0.1mM taurocholic acid in the dorsal OB (Figure 3.16). When examining LFP responses from the 0.001mM pheromone mixture in the dorsal OB, the average peak amplitude during s(-)-uh-301 was enhanced in all of the animals (Figure 3.17). The average peak amplitude of LFP responses showed excitatory effects in all of the animals when examining the 0.1mM amino acid mixture in the lateral OB during s(-)-uh-301 (Figure 3.18). When applied by picospritzer, the vehicle (1% Ethanol) had no effect on the peak amplitude in the dorsal and lateral OB (Figure 3.19). Based on these findings, it is evident that spiperone and s(-)-uh-301 block the modulatory effect caused by 5-HT on the peak amplitude of odour-evoked LFP's in the lateral and dorsal OB and that the 5-HT1a receptor is present in the lamprey OB (Figure 3.20). In an attempt to mask the effect of spiperone blockade of the 5-HT1a receptor by competing with the endogenous ligand, 5-HT, a mixture of 10µM 5-HT plus spiperone was

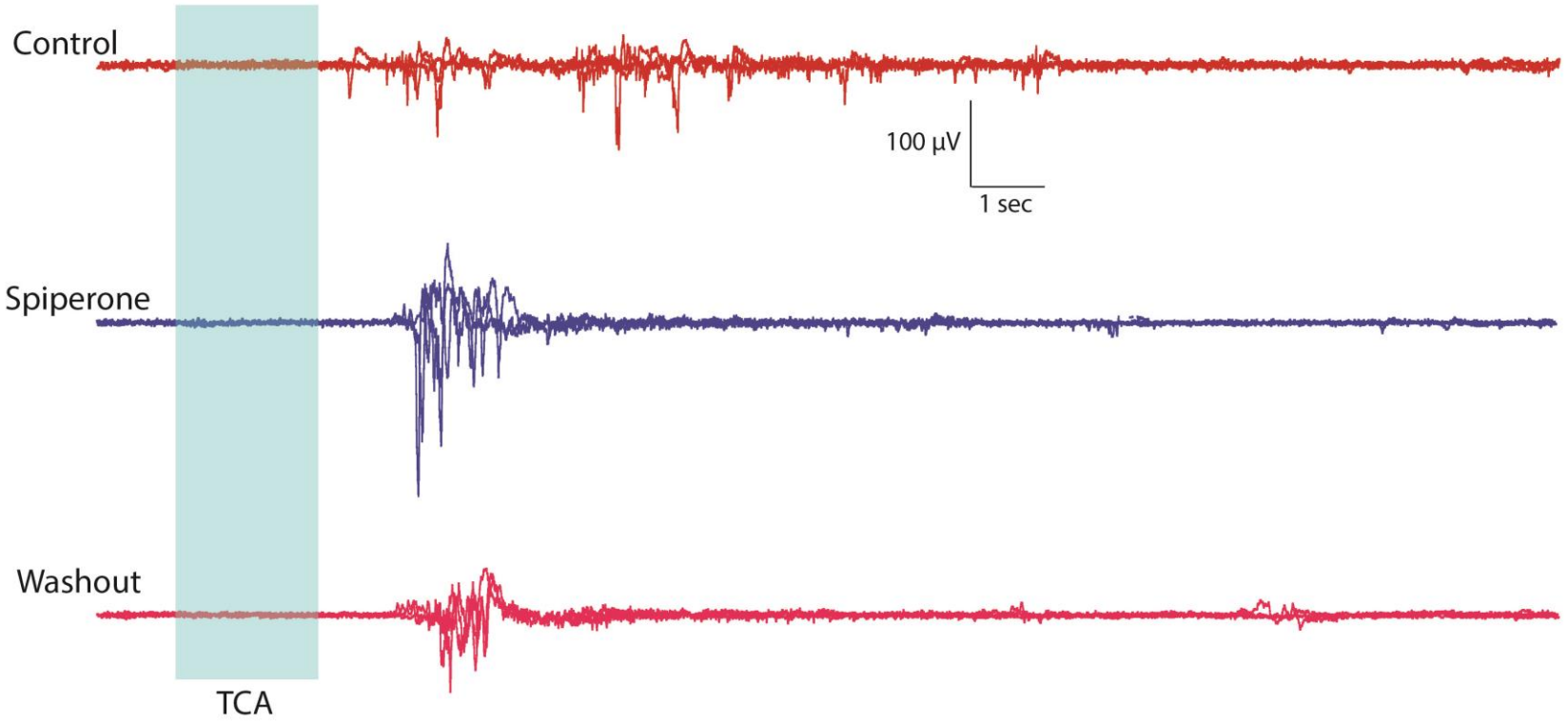


Figure 3.12. Experiment 2: An example of LFP responses in the dorsal OB to 0.1mM TCA showing an excitatory effect on peak amplitude. During the control period (3 superimposed red traces), TCA was applied as an odour test while Ringer's solution was perfused through the recording chamber. The 3 superimposed blue traces represent LFP responses to TCA when spiperone was picospritzed into the olfactory nerve. The 3 superimposed pink traces show LFP activity when TCA was applied during the washout period when spiperone was displaced from the system by Ringer's solution. TCA was applied for 2 seconds (blue shaded box) to the olfactory epithelium and each trace lasted for 20 seconds.

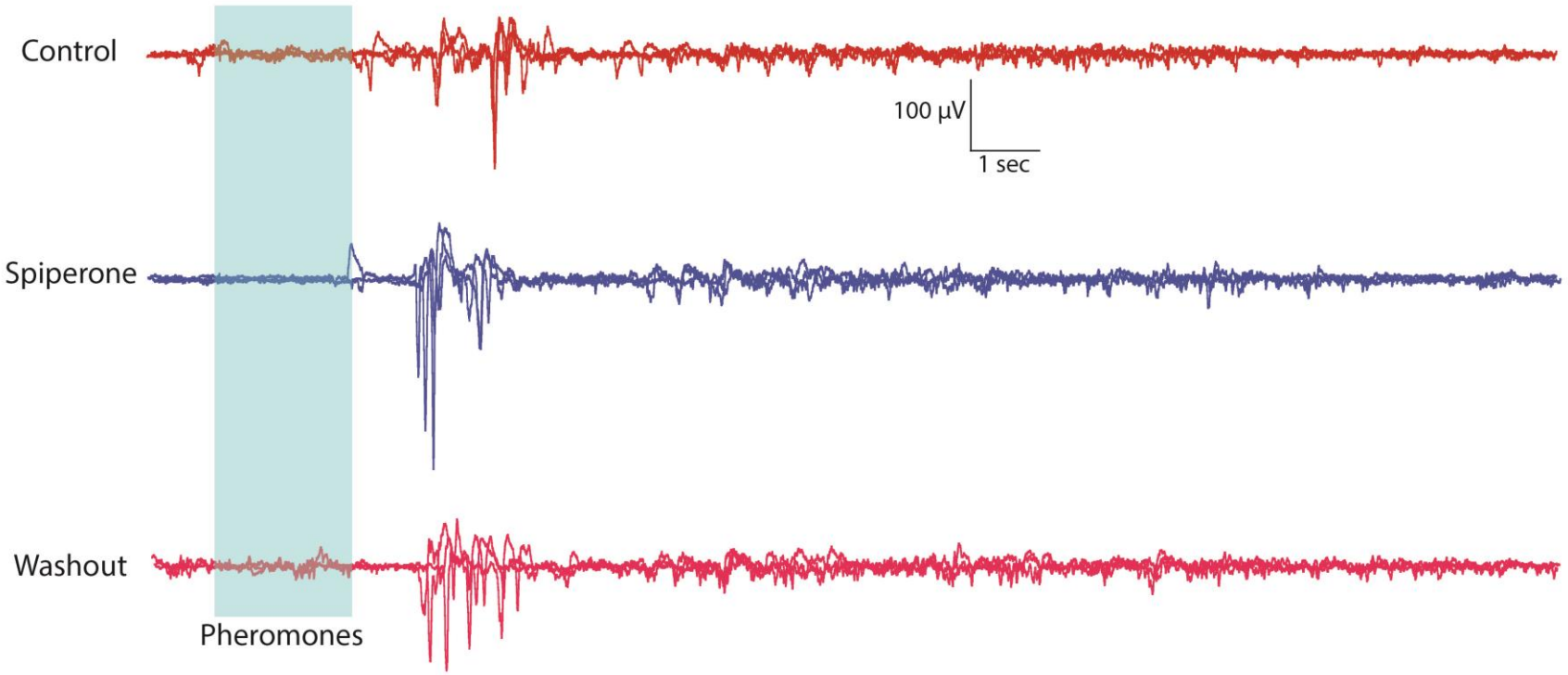


Figure 3.13. Experiment 2: An example of LFP responses in the dorsal OB to a 0.001mM pheromone mixture showing an excitatory effect on peak amplitude. During the control period (3 superimposed red traces), the pheromone mixture was applied as an odour test while Ringer's solution was perfused through the recording chamber. The 3 superimposed blue traces represent LFP responses to the pheromone mixture when spiperone was picospritzed into the olfactory nerve. The 3 superimposed pink traces show LFP activity when the pheromone mixture was applied during the washout period when spiperone was displaced from the system by Ringer's solution. The pheromones were applied for 2 seconds (blue shaded box) to the olfactory epithelium and each trace lasted for 20 seconds.

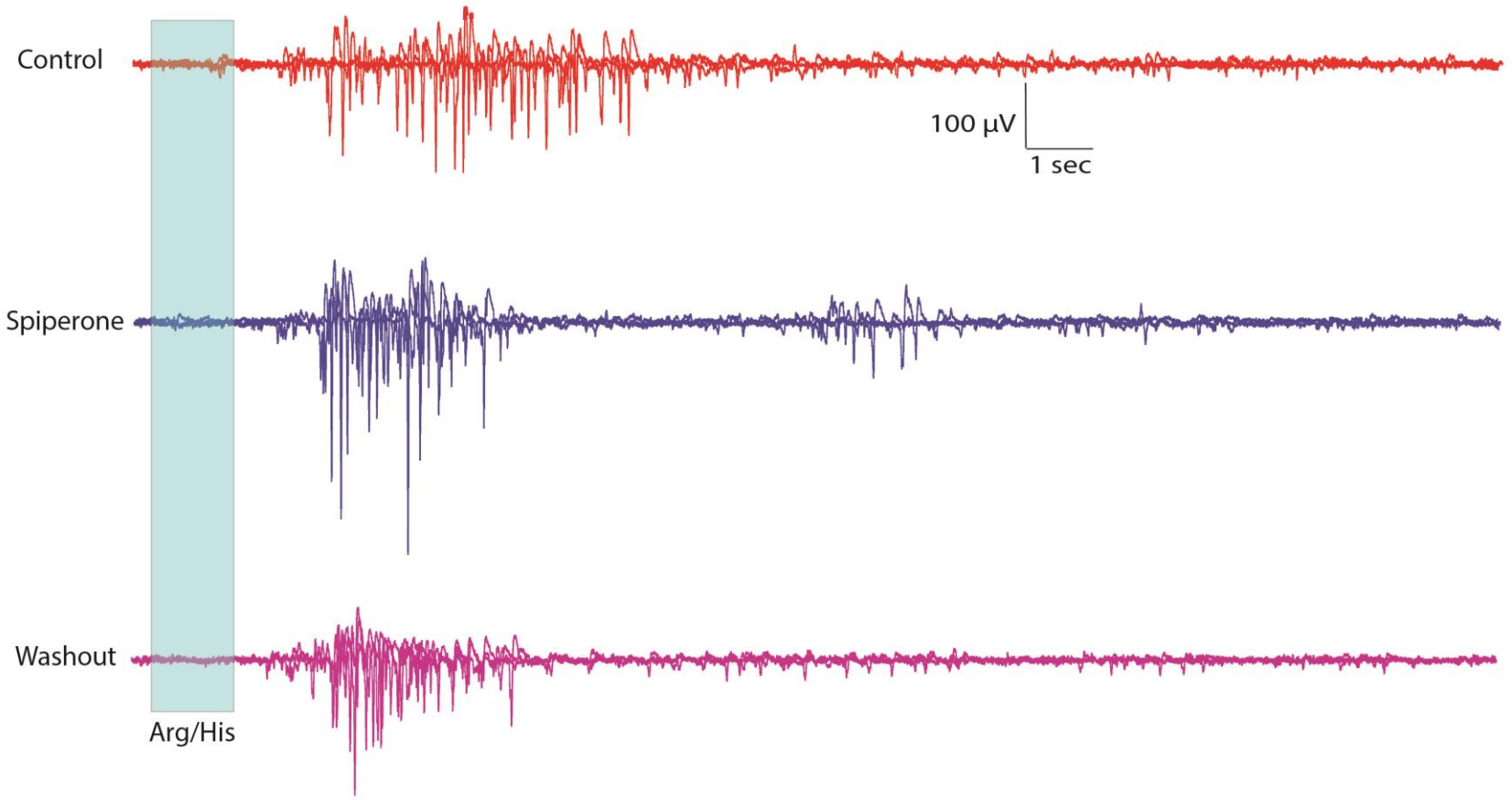


Figure 3.14. Experiment 2: An example of LFP responses in the lateral OB to a 0.1mM amino acid mixture showing an excitatory effect on peak amplitude. During the control period (3 superimposed red traces), the amino acid mixture was applied as an odour test while Ringer's solution was perfused through the recording chamber. The 3 superimposed blue traces represent LFP responses to the amino acid mixture when spiperone was picospritzed into the olfactory nerve. The 3 superimposed pink traces show LFP activity when the amino acid mixture was applied during the washout period when spiperone was displaced from the system by Ringer's solution. The amino acids were applied for 2 seconds (blue shaded box) to the olfactory epithelium and each trace lasted for 20 seconds.

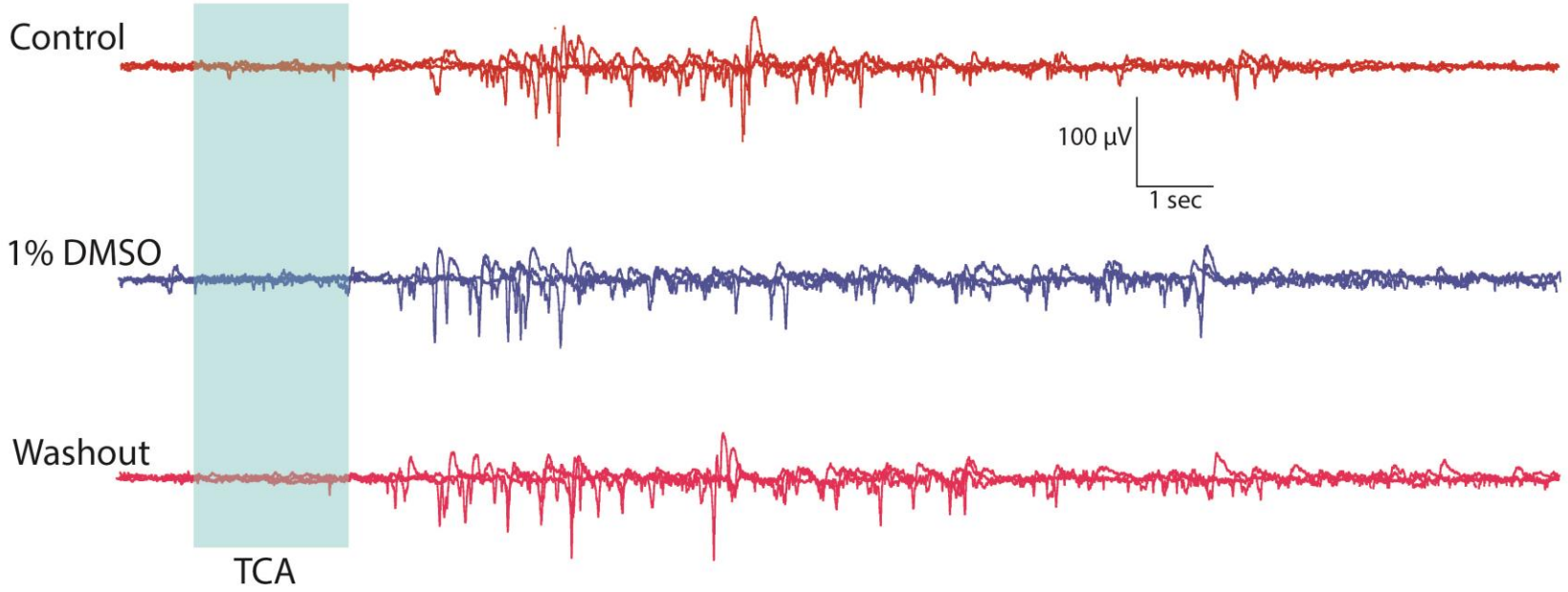


Figure 3.15. Experiment 2: Test for the effect of 1% DMSO on an odour response. An example of LFP responses in the dorsal OB to a 0.1mM TCA solution on peak amplitude when DMSO was picospritzed onto the olfactory nerve. During the control period (3 superimposed red traces), TCA was applied as an odour test while Ringer's solution was perfused through the recording chamber. The 3 superimposed blue traces represent LFP responses to TCA when a 1% DMSO solution was picospritzed into the olfactory nerve. The 3 superimposed pink traces show LFP activity when TCA was applied during the washout period when DMSO was displaced from the system by Ringer's solution. TCA was applied for 2 seconds (blue shaded box) to the olfactory epithelium and each trace lasted for 20 seconds.

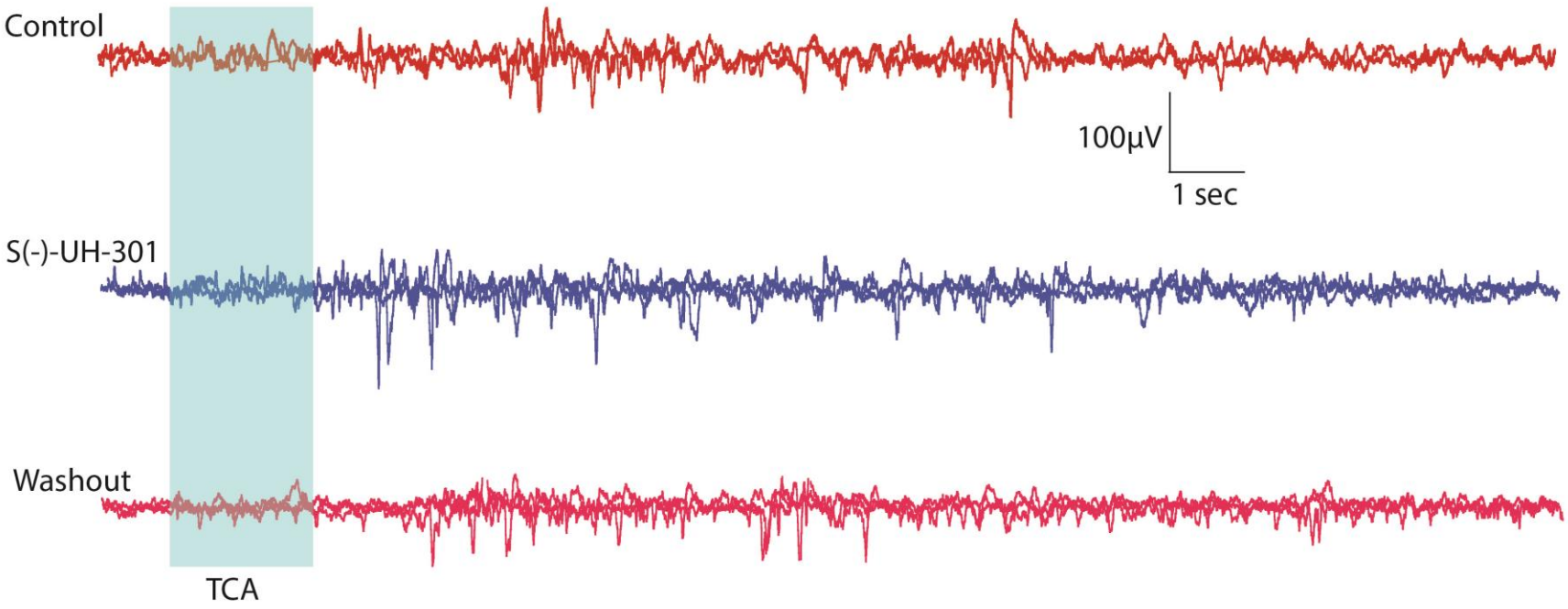


Figure 3.16. Experiment 2: An example of LFP responses in the dorsal OB to the odour 0.1mM TCA during the application of s(-)-uh-301. During the control period (3 superimposed red traces), TCA was applied as an odour test while Ringer's solution was perfused through the recording chamber. The 3 superimposed blue traces represent LFP responses to TCA when s(-)-uh-301 was picospritzed into the olfactory nerve. The 3 superimposed pink traces show LFP activity when TCA was applied during the washout period when s(-)-uh-301 was displaced from the system by Ringer's solution. TCA was applied for 2 seconds (blue shaded box) to the olfactory epithelium and each trace lasted for 20 seconds.

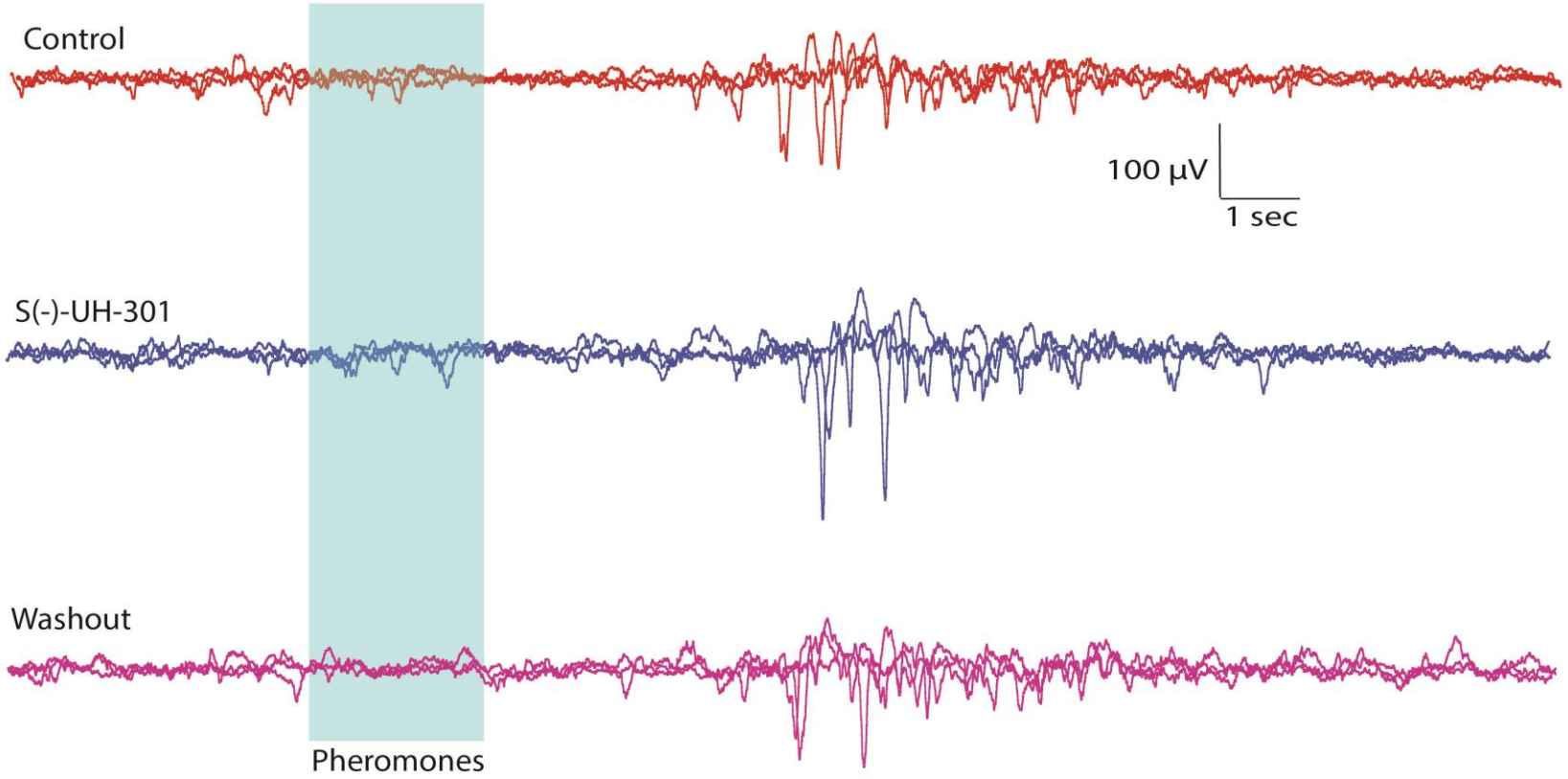


Figure 3.17. Experiment 2: An example of LFP responses in the dorsal OB to a 0.001mM pheromone mixture during the application of s(-)-uh-301. During the control period (3 superimposed red traces), the pheromone mixture was applied as an odour test while Ringer's solution was perfused through the recording chamber. The 3 superimposed blue traces represent LFP responses to the pheromone mixture when s(-)-uh-301 was picospritzed into the olfactory nerve. The 3 superimposed pink traces show LFP activity when the pheromone mixture was applied during the washout period when s(-)-uh-301 was displaced from the system by Ringer's solution. The pheromones were applied for 2 seconds (blue shaded box) to the olfactory epithelium and each trace lasted for 20 seconds.

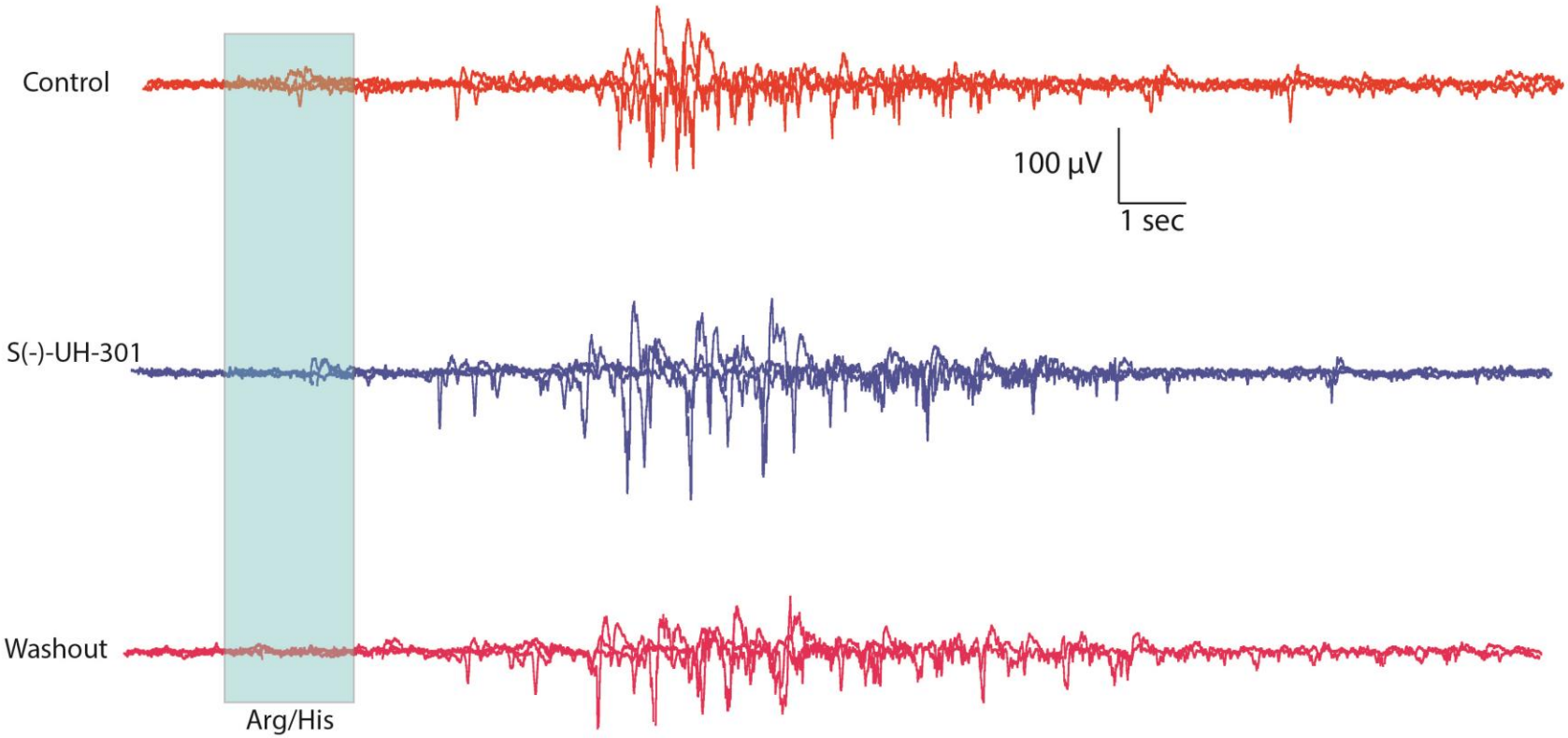


Figure 3.18. Experiment 2: An example of LFP responses in the lateral OB to a 0.1mM amino acid mixture during the application of s(-)-uh-301. During the control period (3 superimposed red traces), the amino acid mixture was applied as an odour test while Ringer's solution was perfused through the recording chamber. The 3 superimposed blue traces represent LFP responses to the amino acid mixture when s(-)-uh-301 was picospritzed into the olfactory nerve. The 3 superimposed pink traces show LFP activity when the amino acid mixture was applied during the washout period when s(-)-uh-301 was displaced from the system by Ringer's solution. The amino acids were applied for 2 seconds (blue shaded box) to the olfactory epithelium and each trace lasted for 20 seconds.

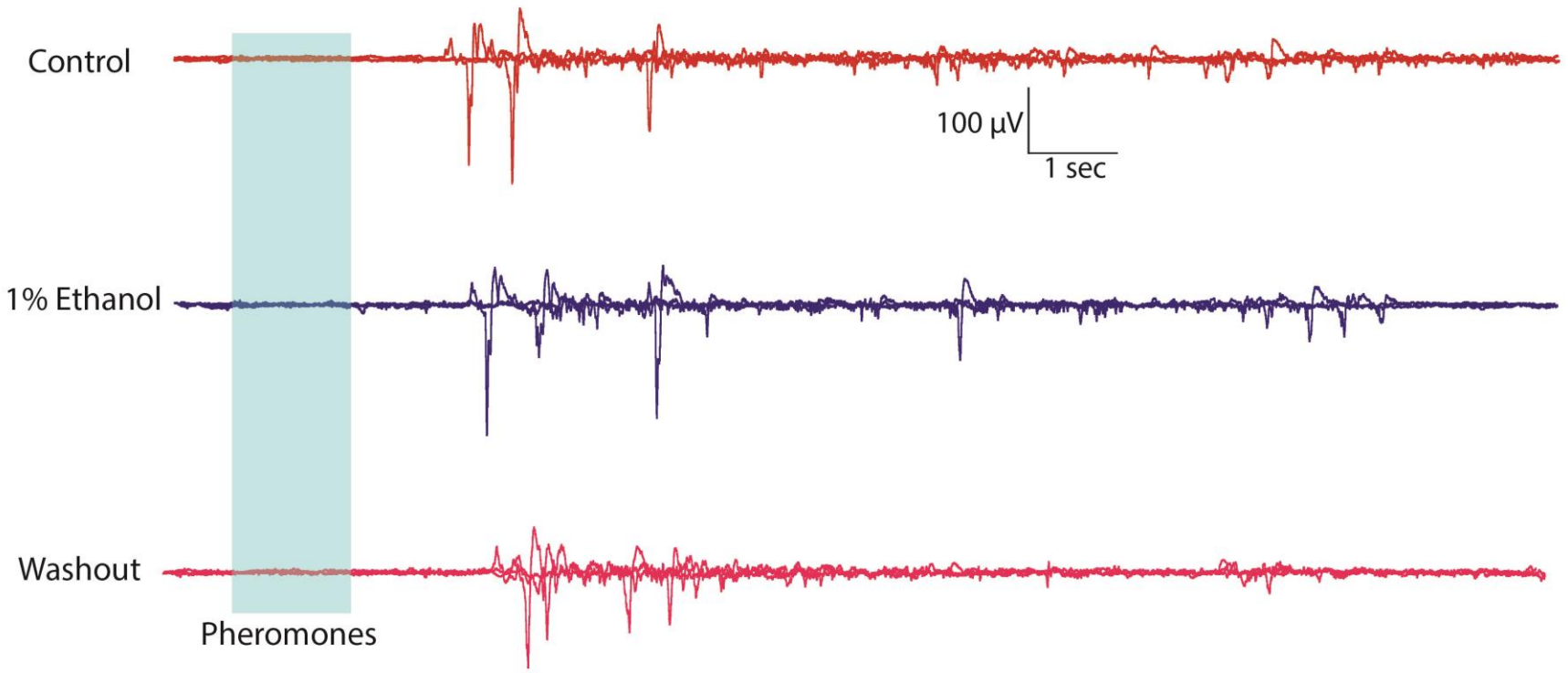
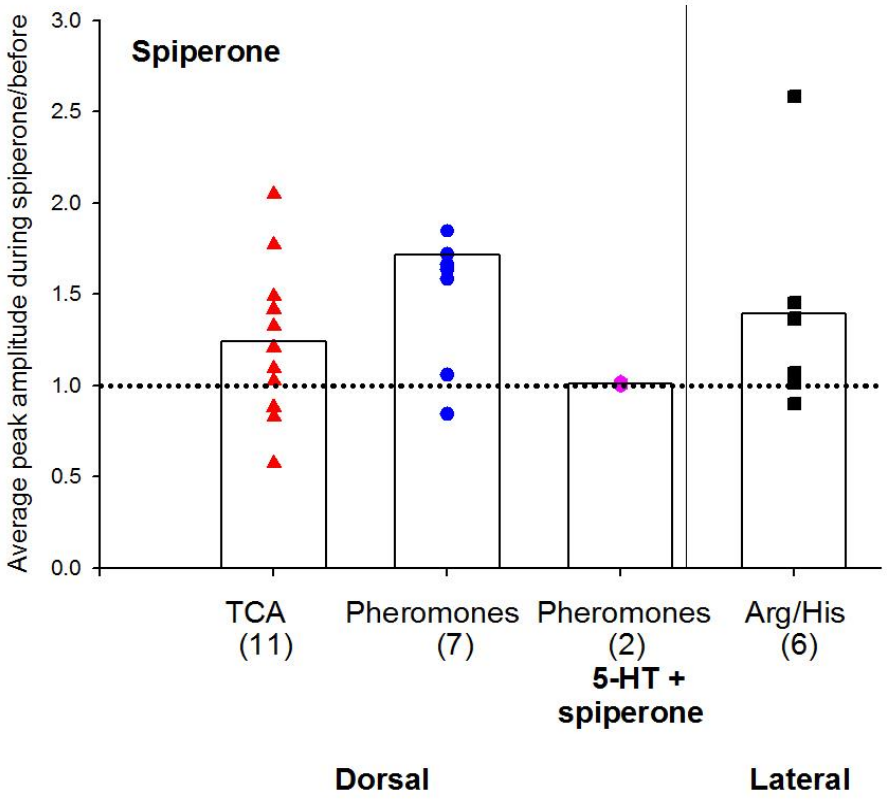


Figure 3.19. Experiment 2: An example of LFP responses in the dorsal OB to a 0.001mM pheromone mixture during the application of 1% ethanol. During the control period (3 superimposed red traces), the pheromone mixture was applied as an odour test while Ringer's solution was perfused through the recording chamber. The 3 superimposed blue traces represent LFP responses to the pheromone mixture when a 1% ethanol solution was picospritzed into the olfactory nerve. The 3 superimposed pink traces show LFP activity when the pheromone mixture was applied during the washout period when ethanol was displaced from the system by Ringer's solution. The pheromones were applied for 2 seconds (blue shaded box) to the olfactory epithelium and each trace lasted for 20 seconds.

A


Spiperone
Average peak amplitude during spiperone/before
3.0
2.5
2.0
1.5
1.0
0.5
0.0
TCA (11)
Pheromones (7)
Pheromones (2) 5-HT + spiperone
Arg/His (6)
Dorsal
Lateral

B

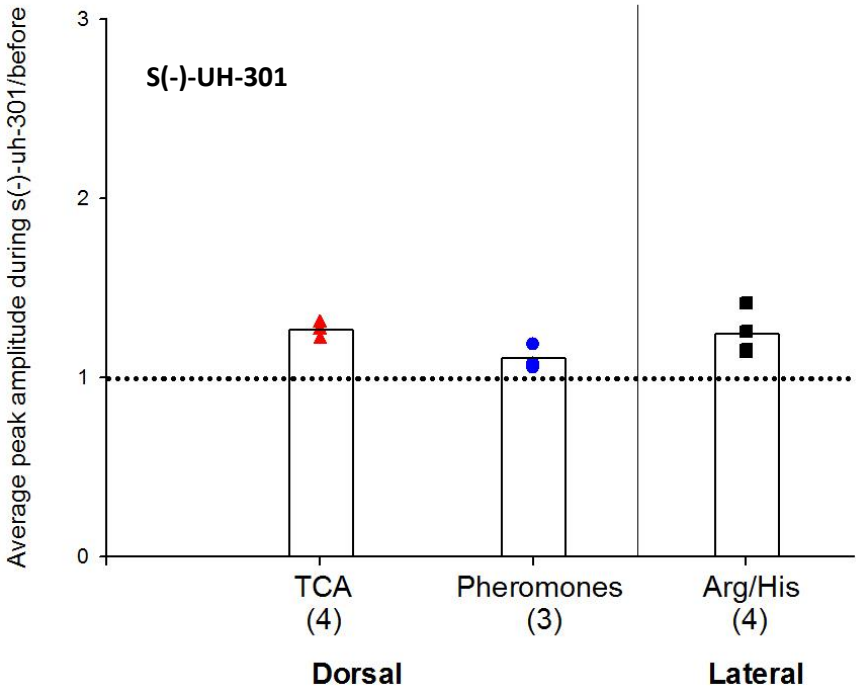

S(-)-UH-301
Average peak amplitude during s(-)-uh-301/before
3
2
1
0
TCA (4)
Pheromones (3)
Arg/His (4)
Dorsal
Lateral

Figure 3.20. Experiment 2: The effect of the 5-HT1a antagonists, spiperone (A) and s(-)-uh-301 (B), on the peak amplitude (μV) of LFP odour-evoked responses in the dorsal and lateral olfactory bulb (OB). The x-axis represents the tested odours with the number of animals tested of each in brackets. The solid line separates the two different regions of the OB in which the odours were tested. The y-axis represents the value calculated from the ratio of the average peak amplitude when the antagonists were in the OB divided by the average peak amplitude before the antagonists were applied. Each point on the graph symbolizes one animal and each odour is shown by a different colour and shape. If a point is below the dashed line (<1), then it is considered inhibitory. If a point is above the dashed line (>1), then it is considered excitatory. The bar graph represents the standard mean of the response magnitude for each odour.

picospritzed into the ON during a pheromone application. Since 5-HT shows an inhibitory effect on peak amplitude and spiperone causes an enhanced effect, applying a mixture containing both 5-HT and spiperone should result in no change on the peak amplitude. The 5-HT plus antagonist mixture caused no effect on the peak amplitude in the dorsal (Figure 3.21) or lateral OB. This finding supports the idea that 5-HT and spiperone compete for the 5-HT1a receptor. When picospritzing directly into the OB, s(-)-uh-301 caused the peak amplitude of taurocholic acid to be enhanced (Figure 3.22).

3.4 Experiment 2: Effect of picospritzing the 5-HT1a antagonists and 5-HT + antagonist on temporal aspects of odour-evoked local field potential responses.

When examining response duration of TCA during the application of 10μM spiperone in the dorsal OB, responses were longer 5 out of 11 preparations, shorter 5 preparations and unchanged in 1 preparation (Figure 3.23). When examining response duration of pheromones during the application of spiperone in the dorsal OB, 3 out of 7 preparations displayed a shorter response, 3 were longer and 1 was unchanged (Figure 3.23). When examining response duration of amino acids during the application of spiperone in the lateral OB, 5 out of 6 animals had a longer response and 1 saw a shorter response (Figure 3.23). When examining TCA responses in the dorsal OB, the peak number increased in 7 out of 11 preparations and decreased in 4 preparations during the application of spiperone (Figure 3.24). When examining pheromone responses in the dorsal OB, the number of peaks increased in 3 out of 7 preparations, decreased in 3 preparations and was unchanged in 1 preparation during the application of spiperone (Figure 3.24). When examining amino acid responses in the lateral OB, the number of peaks increased in all preparations during the application of spiperone (Figure 3.24). Therefore, the effect of spiperone on 5-HT modulation of the response duration and the number of peaks for pheromones and taurocholic acid is unclear, while there is a clear effect on the temporal

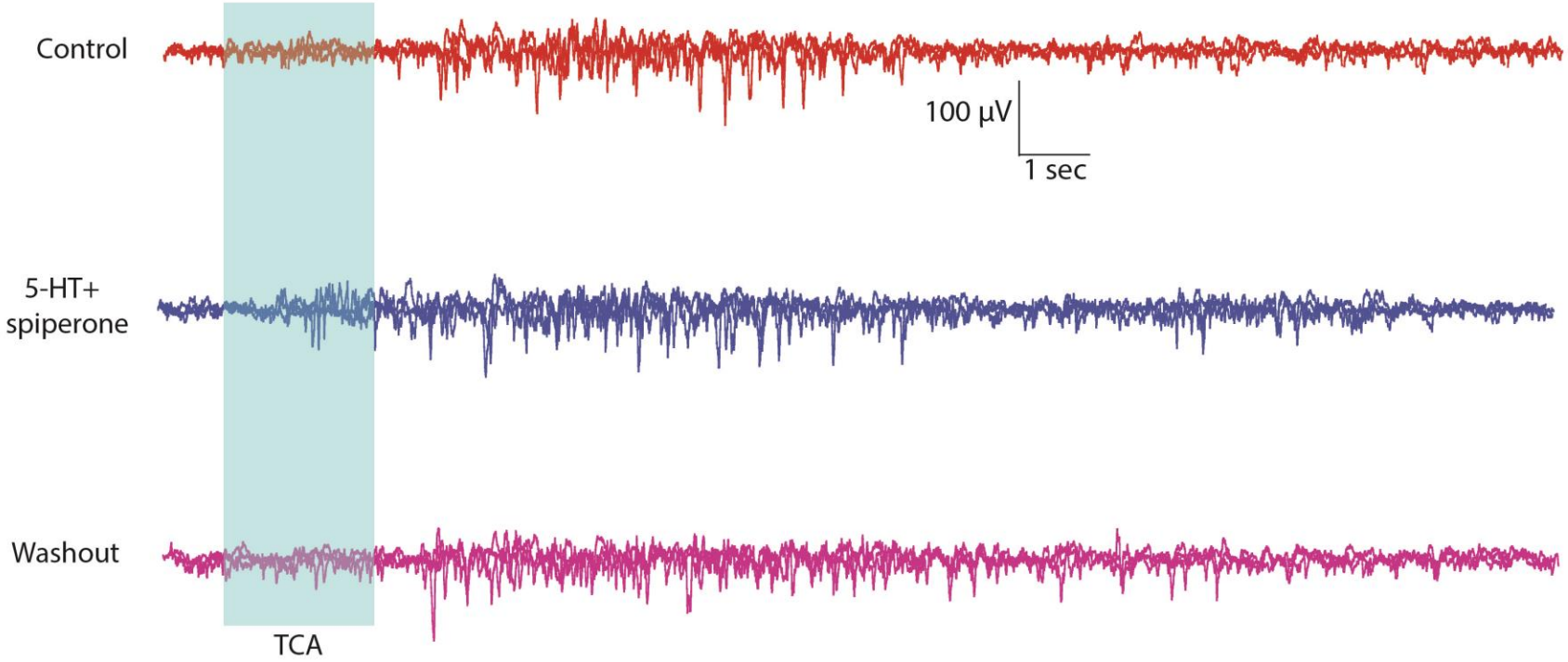


Figure 3.21. Experiment 2: An example of LFP responses in the dorsal OB to 0.1mM TCA during the application of 5-HT and spiperone. During the control period (3 superimposed red traces), TCA was applied as an odour test while Ringer's solution was perfused through the recording chamber. The 3 superimposed blue traces represent LFP responses to TCA when a mixture of 5-HT and spiperone was picospritzed into the olfactory nerve. The 3 superimposed pink traces show LFP activity when TCA was applied during the washout period when the 5-HT and spiperone mixture was displaced from the system by Ringer's solution. TCA was applied for 2 seconds (blue shaded box) to the olfactory epithelium and each trace lasted for 20 seconds.

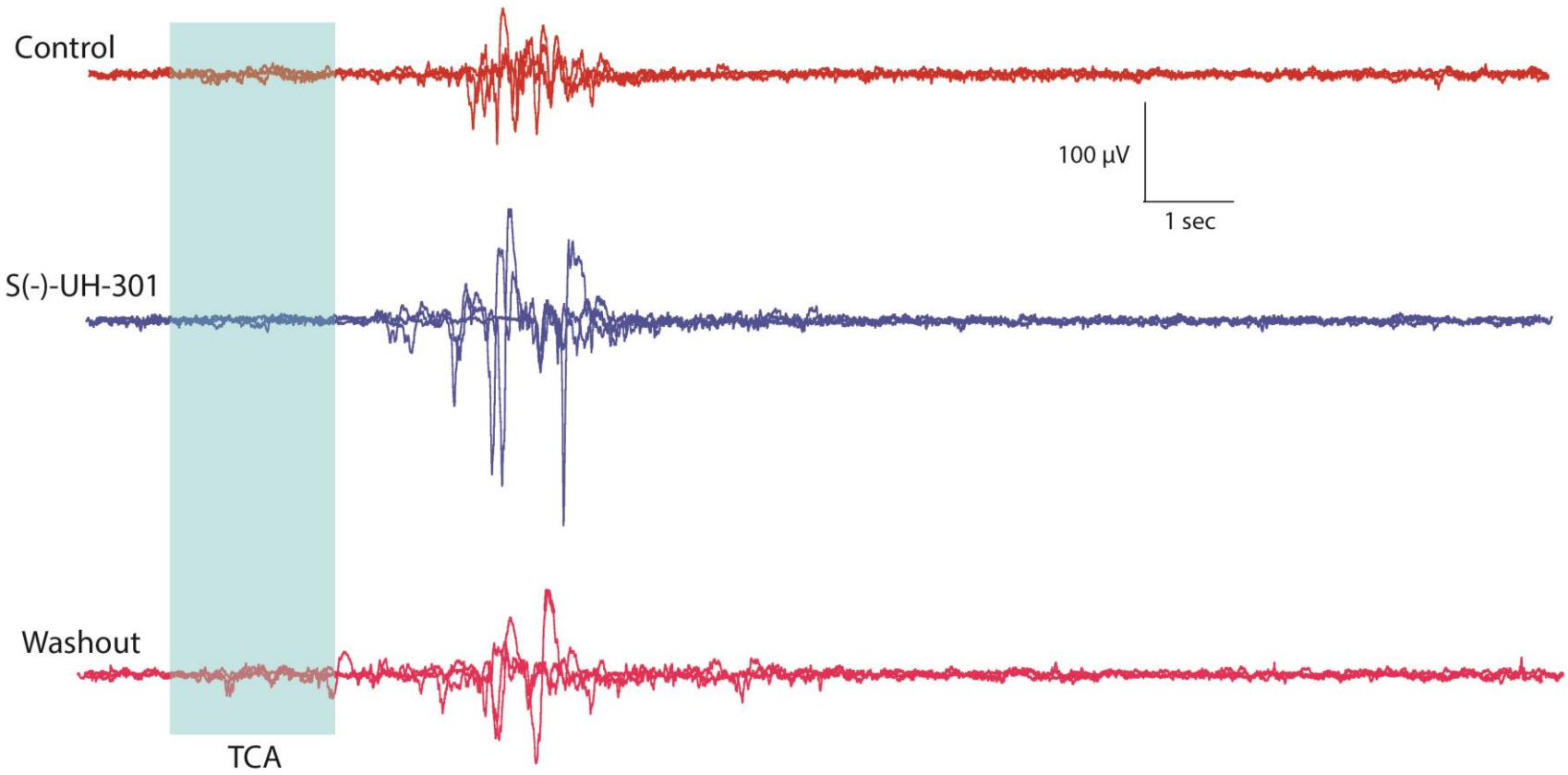


Figure 3.22. Experiment 2: An example of LFP responses in the dorsal OB to 0.1mM TCA during the application of s(-)-uh-301 directly into the OB. During the control period (3 superimposed red traces), TCA was applied as an odour test while Ringer's solution was perfused through the recording chamber. The 3 superimposed blue traces represent LFP responses to TCA when s(-)-uh-301 was picospritzed into the olfactory bulb. The 3 superimposed pink traces show LFP activity when TCA was applied during the washout period when s(-)-uh-301 was displaced from the system by Ringer's solution. TCA was applied for 2 seconds (blue shaded box) to the olfactory epithelium and each trace lasted for 20 seconds

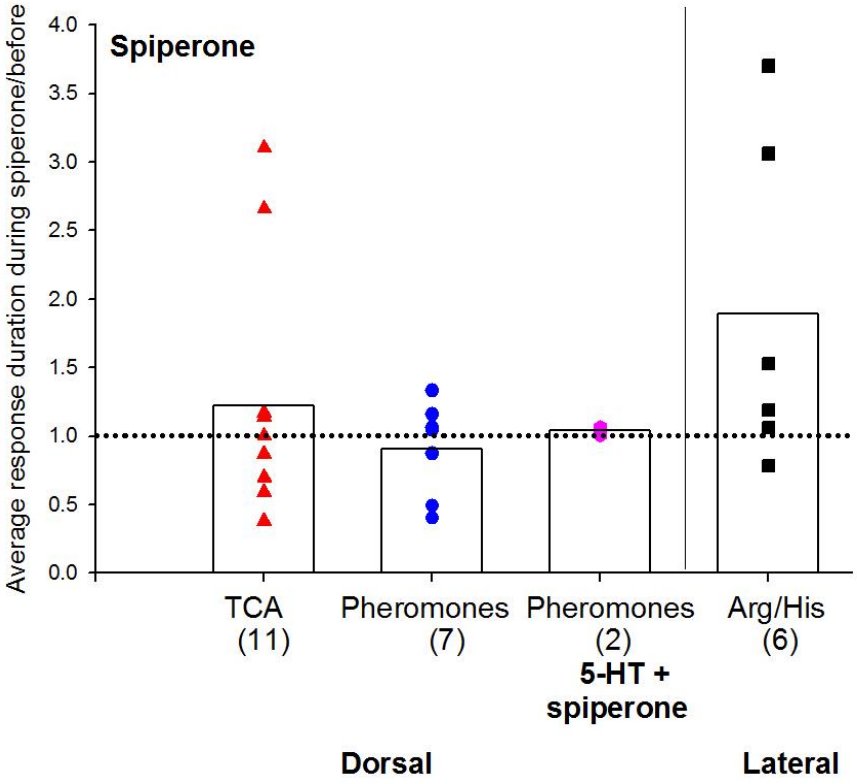
parameters of amino acids in the lateral OB by lengthening responses and increasing the number of peaks.

When investigating the effect of picospritzing 5-HT plus spiperone into the olfactory nerve (Figure 3.20), there was no change in response duration or number of peaks. This finding supports the idea that 5-HT competing with spiperone for the 5-HT1a receptor blocked the excitatory effects caused by spiperone.

When examining response duration of TCA responses during the application of 1µM s(-)-uh-301 in the dorsal OB, responses were longer in all preparations (Figure 3.23). When examining response duration of pheromones during the application of s(-)-uh-301 in the dorsal OB, 2 out of 3 preparations displayed a longer response while 1 was shorter (Figure 3.23). When examining response duration of amino acids during the application of s(-)-uh-301 in the lateral OB, 3 out of 4 animals had a longer response while 1 saw a shorter response (Figure 3.23). When examining TCA responses in the dorsal OB, the peak number increased in 3 out of 4 preparations and 1 saw a decrease during the application of s(-)-uh-301 (Figure 3.24). When examining pheromone responses in the dorsal OB, the number of peaks increased in all of the preparations during the application of s(-)-uh-301 (Figure 3.24). When examining amino acid responses in the lateral OB, the number of peaks increased in 3 out of 4 preparations, while 1 preparation saw a decrease during the application of s(-)-uh-301 (Figure 3.24).

The application of 1% DMSO caused no change on the peak amplitude, response duration or the number of peaks of odour responses (Figure 3.25). The application of 1% ethanol resulted in no change on the peak amplitude, response duration or the number of peaks of odour responses (Figure 3.26).

A


Spiperone
Average response duration during spiperone/before
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
TCA (11)
Pheromones (7)
Pheromones (2) 5-HT + spiperone
Arg/His (6)
Dorsal
Lateral

B

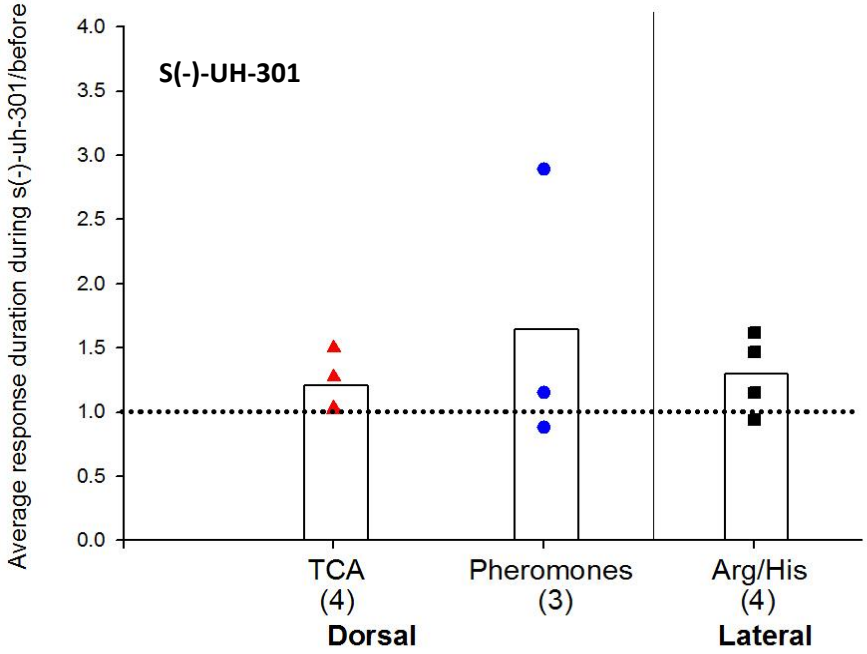
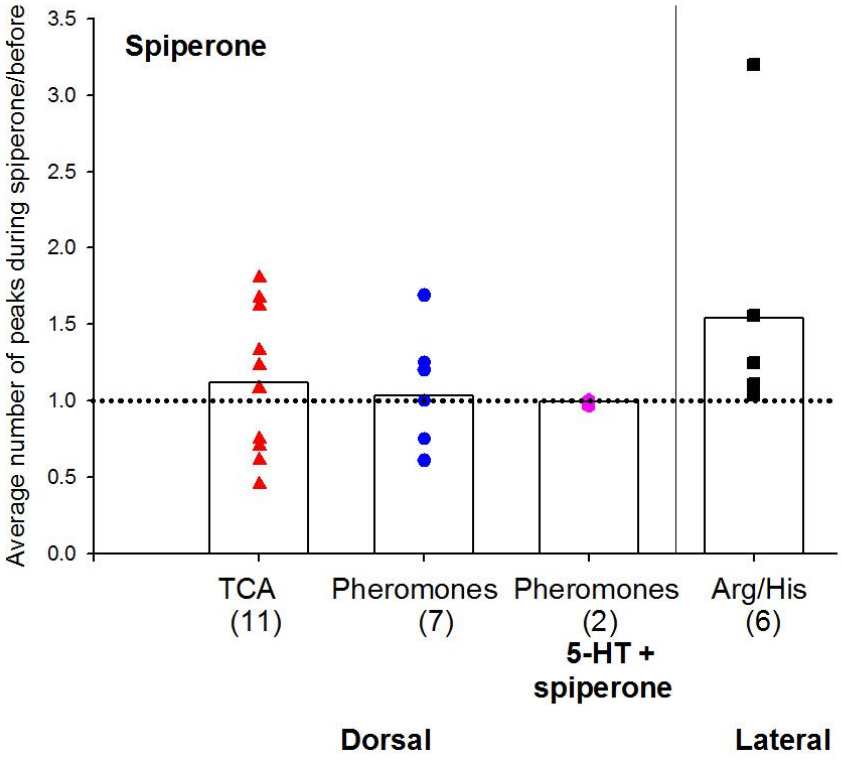

S(-)-UH-301
Average response duration during s(-)-uh-301/before
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
TCA (4)
Pheromones (3)
Arg/His (4)
Dorsal
Lateral

Figure 3.23. Experiment 2: The effect of the 5-HT1a antagonists, spiperone (A) and s(-)-uh-301 (B), on the response duration (ms) of LFP odour-evoked responses in the dorsal and lateral olfactory bulb (OB). The x-axis represents the tested odours with the number of animals tested of each in brackets. The solid line separates the two different regions of the OB in which the odours were tested. The y-axis represents the value calculated from the ratio of the average response duration when the antagonists were in the OB divided by the average response duration before the antagonists were applied. Each point on the graph symbolizes one animal and each odour is shown by a different colour and shape. If a point is below the dashed line (<1), then it is considered inhibitory. If a point is above the dashed line (>1), then it is considered excitatory. The bar graph represents the standard mean of the response magnitude for each odour.

A


Spiperone
Average number of peaks during spiperone/before
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
TCA (11)
Pheromones (7)
Pheromones (2) 5-HT + spiperone
Arg/His (6)
Dorsal
Lateral

B

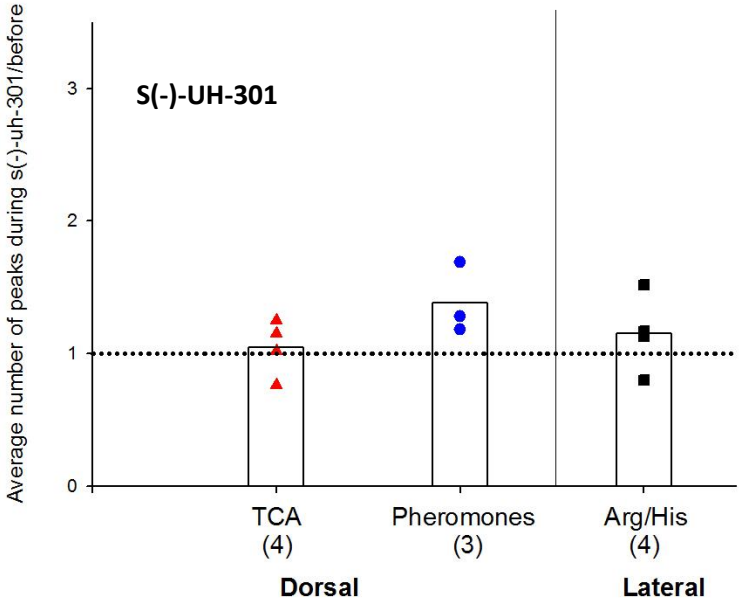

S(-)-UH-301
Average number of peaks during s(-)-uh-301/before
3
2
1
0
TCA (4)
Pheromones (3)
Arg/His (4)
Dorsal
Lateral

Figure 3.24. Experiment 2: The effect of the 5-HT1a antagonists, spiperone (A) and s(-)-uh-301 (B), on the number of peaks in LFP odour-evoked responses in the dorsal and lateral olfactory bulb (OB). The x-axis represents the tested odours with the number of animals tested of each in brackets. The solid line separates the two different regions of the OB in which the odours were tested. The y-axis represents the value calculated from the ratio of the average peak amplitude when the antagonists were in the OB divided by the average peak amplitude before the antagonists were applied. Each point on the graph symbolizes one animal and each odour is shown by a different colour and shape. If a point is below the dashed line (<1), then it is considered inhibitory. If a point is above the dashed line (>1), then it is considered excitatory. The bar graph represents the standard mean of the response magnitude for each odour.

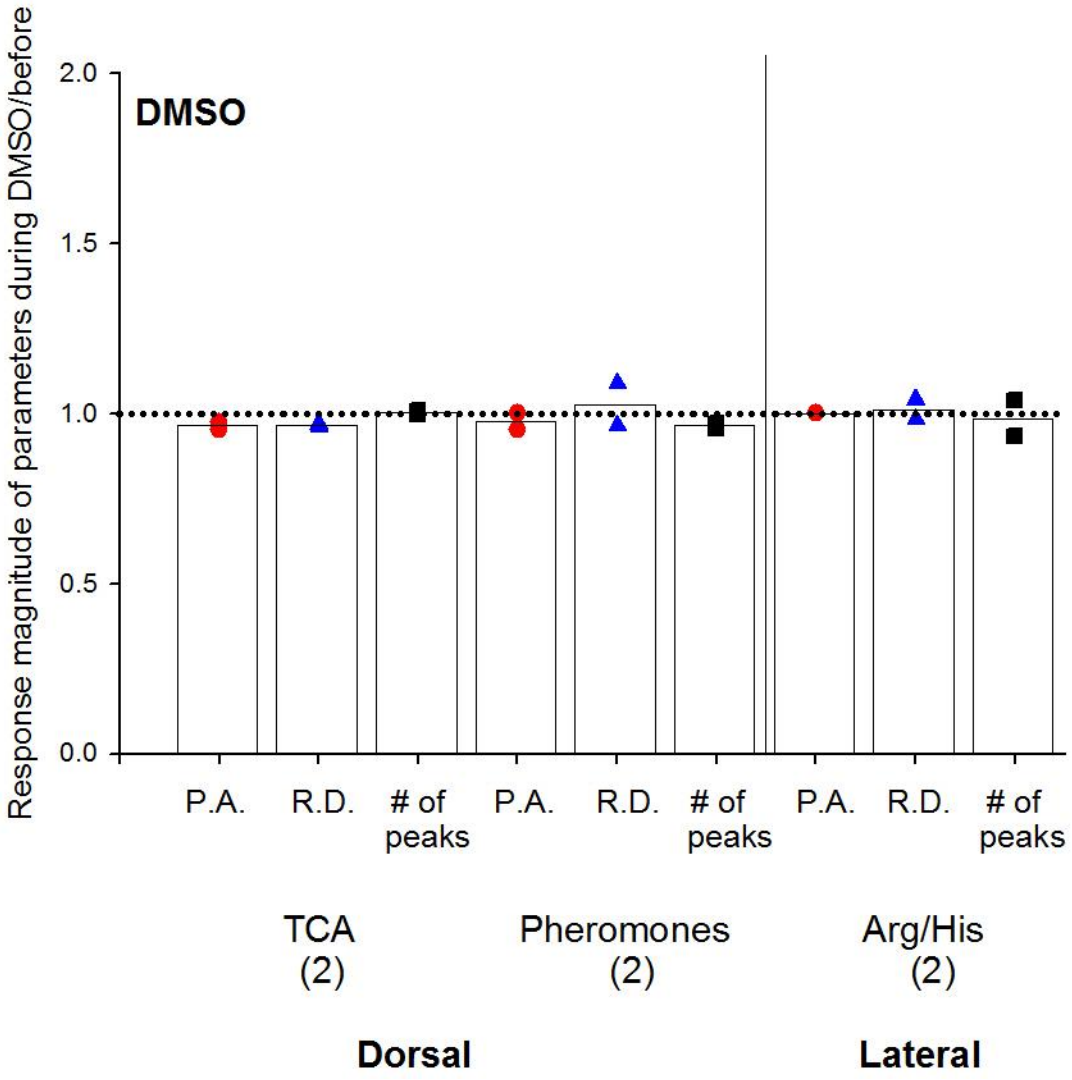


Figure 3.25. Experiment 2: The effect of the vehicle blank, DMSO, on the number of peaks, response duration and peak amplitude of LFP odour-evoked responses in the dorsal and lateral olfactory bulb (OB). The x-axis represents the parameters for each odour tested with the number of animals tested of each in brackets. The solid line separates the two different regions of the OB in which the odours were tested. The y-axis represents the value calculated from the ratio of the average peak amplitude when the antagonists were in the OB divided by the average peak amplitude before the antagonists were applied. Each point on the graph symbolizes one animal and each odour is shown by a different colour and shape. If a point is below the dashed line (<1), then it is considered inhibitory. If a point is above the dashed line (>1), then it is considered excitatory. The bar graph represents the standard mean of the response magnitude of each parameter for each odour.

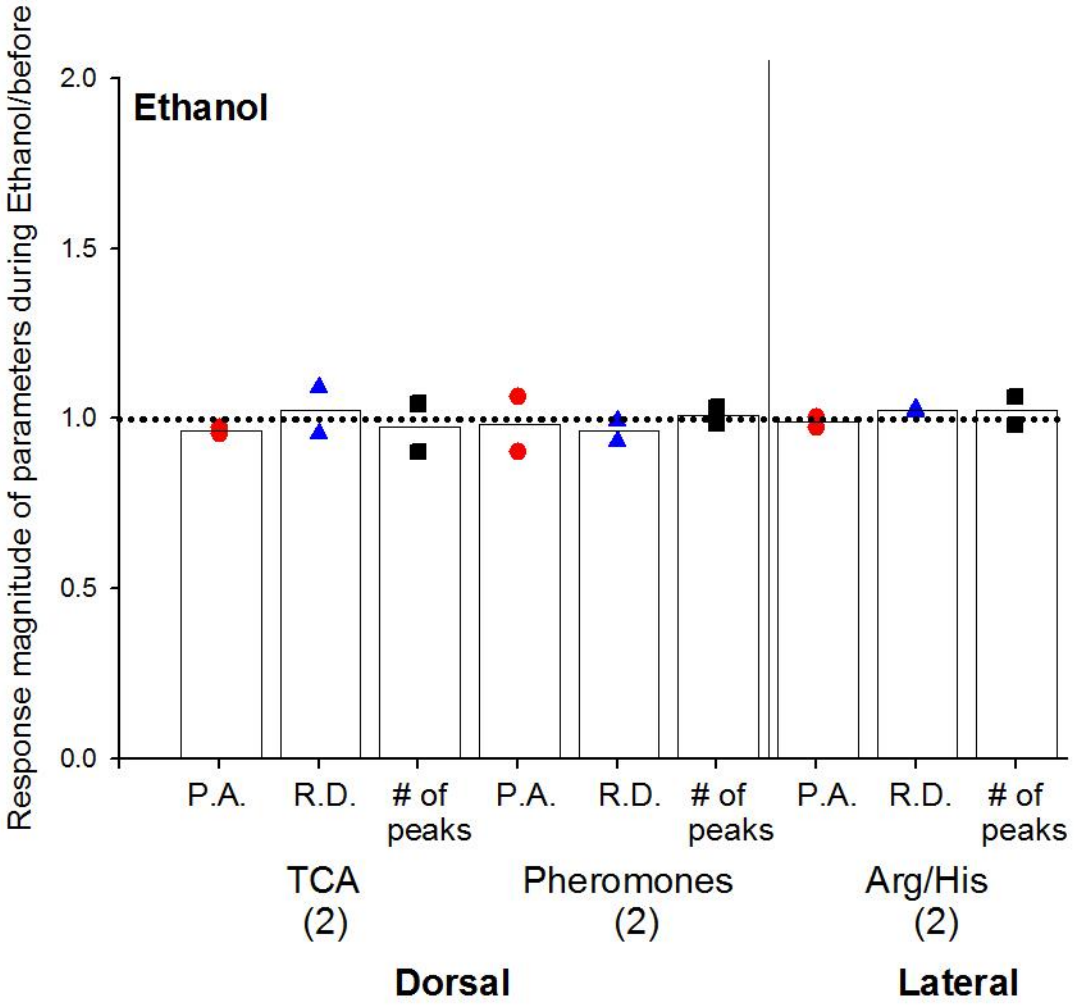


Figure 3.26. Experiment 2: The effect of the vehicle blank, ethanol, on the number of peaks, response duration and peak amplitude of LFP odour-evoked responses in the dorsal and lateral olfactory bulb (OB). The x-axis represents the parameters for each odour tested with the number of animals tested of each in brackets. The solid line separates the two different regions of the OB in which the odours were tested. The y-axis represents the value calculated from the ratio of the average peak amplitude when the antagonists were in the OB divided by the average peak amplitude before the antagonists were applied. Each point on the graph symbolizes one animal and each odour is shown by a different colour and shape. If a point is below the dashed line (<1), then it is considered inhibitory. If a point is above the dashed line (>1), then it is considered excitatory. The bar graph represents the standard mean of the response magnitude of each parameter for each odour.

Chapter 4
Discussion

The present study investigated the effect of 5-HT modulation through bath application and focal application by picospritzing of the 5-HT1a antagonists, spiperone and s(-)-uh-301, on odour-evoked neural responses in the OB using a model species, the sea lamprey. In experiment 1, 5-HT attenuated the peak amplitude of amino acid responses in the dorsal and lateral regions of the OB (Figure 4.1), but the effect was less clear for pheromones and TCA responses in the dorsal OB. In experiment 2, pharmacological blockade of the 5-HT1a receptor enhanced the peak amplitude of all odour responses in the lateral and dorsal regions of the OB (Figure 4.1).

In experiment 1, the effect of 5-HT on the temporal parameters of response duration and the number of peaks was unclear (Figure 4.2). In experiment 2, the effect of the 5-HT1a antagonist, spiperone, on the temporal parameters of pheromones and taurocholic acid in the dorsal OB was unclear, but it had a modulatory effect on amino acids in the lateral OB (Figure 4.2). The other 5-HT1a antagonist, s(-)-uh-301, had modulatory effects on the temporal patterns in all odours in both regions of the OB (Figure 4.2).

These results are consistent with 5-HT modulation on olfaction in other vertebrate species (Baumgarten et al., 1963; Bloom et al., 1964; McLean et al., 1993 Langdon et al., 1997; Hardy et al., 2005; Petzold et al., 2009; Ganesh et al., 2010) and show the opposite effect of 5-HT modulation on olfaction in invertebrate species (Dacks et al., 2008).

When examining peak amplitude of odour-evoked LFP's, we were recording the collective depolarization of a large group of neurons in responses to odours. In experiment 1, the effect of 5-HT bath application predominantly caused an inhibitory effect on peak amplitude. This is significant because it is consistent with the normal inhibitory mechanism of action of the 5-HT1a receptor (see section 1.6.1). This means that when 5-HT bound to 5-HT1a receptors in the OB of the lamprey, the 5-HT caused hyperpolarization of the neurons in the OB from

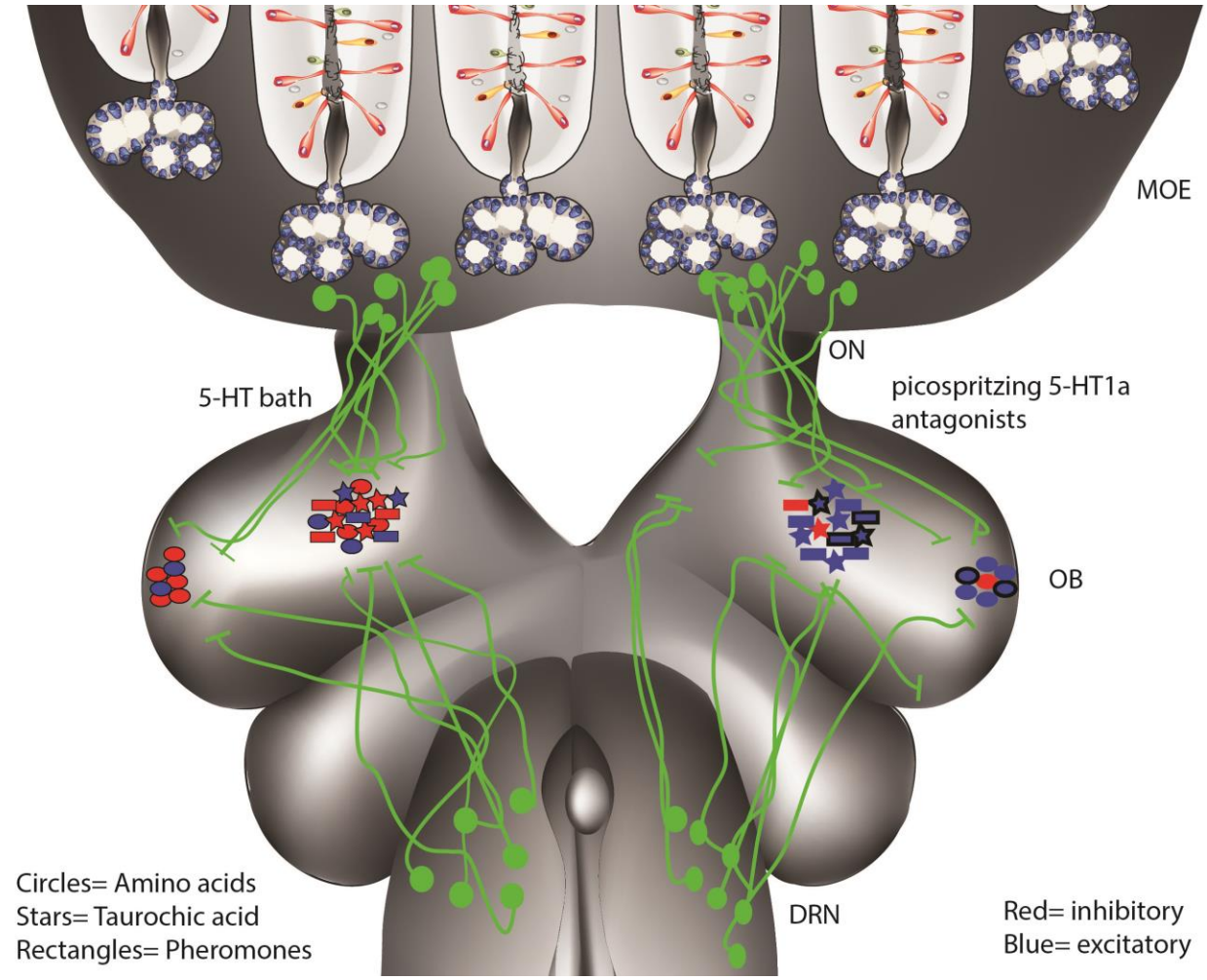


Figure 4.1. A summary diagram of the effect of 5-HT bath application and picospritzing of 5-HT1a antagonists on peak amplitude of odour-evoked LFP responses in the olfactory bulb (OB). The 5-HT bath application (left OB) mostly caused inhibitory effects (red) on the peak amplitude of amino acids (circles), pheromones (rectangles) and taurocholic acid (stars) in the dorsal OB and of amino acids in the lateral OB. However, the 5-HT bath also caused excitatory effects (blue) on the peak amplitude in a few recordings for every odour in both regions of the OB. Picospritzing (right bulb) of the 5-HT1a antagonist, s(-)-uh-301 (black outline), always caused an excitatory effect on the peak amplitude of pheromones and taurocholic acid in the dorsal OB and amino acids in the lateral OB. Picospritzing of the 5-HT1a antagonist, spiperone hydrochloride (no outline), caused an excitatory effect in most of the recordings on peak amplitude of taurocholic acid and pheromones in the dorsal OB and amino acids in the lateral OB. Spiperone also caused inhibitory effects in a few recordings on all odours in both regions of the OB. DRN-Dorsal Raphe Nuclei; MOE-main olfactory epithelium.

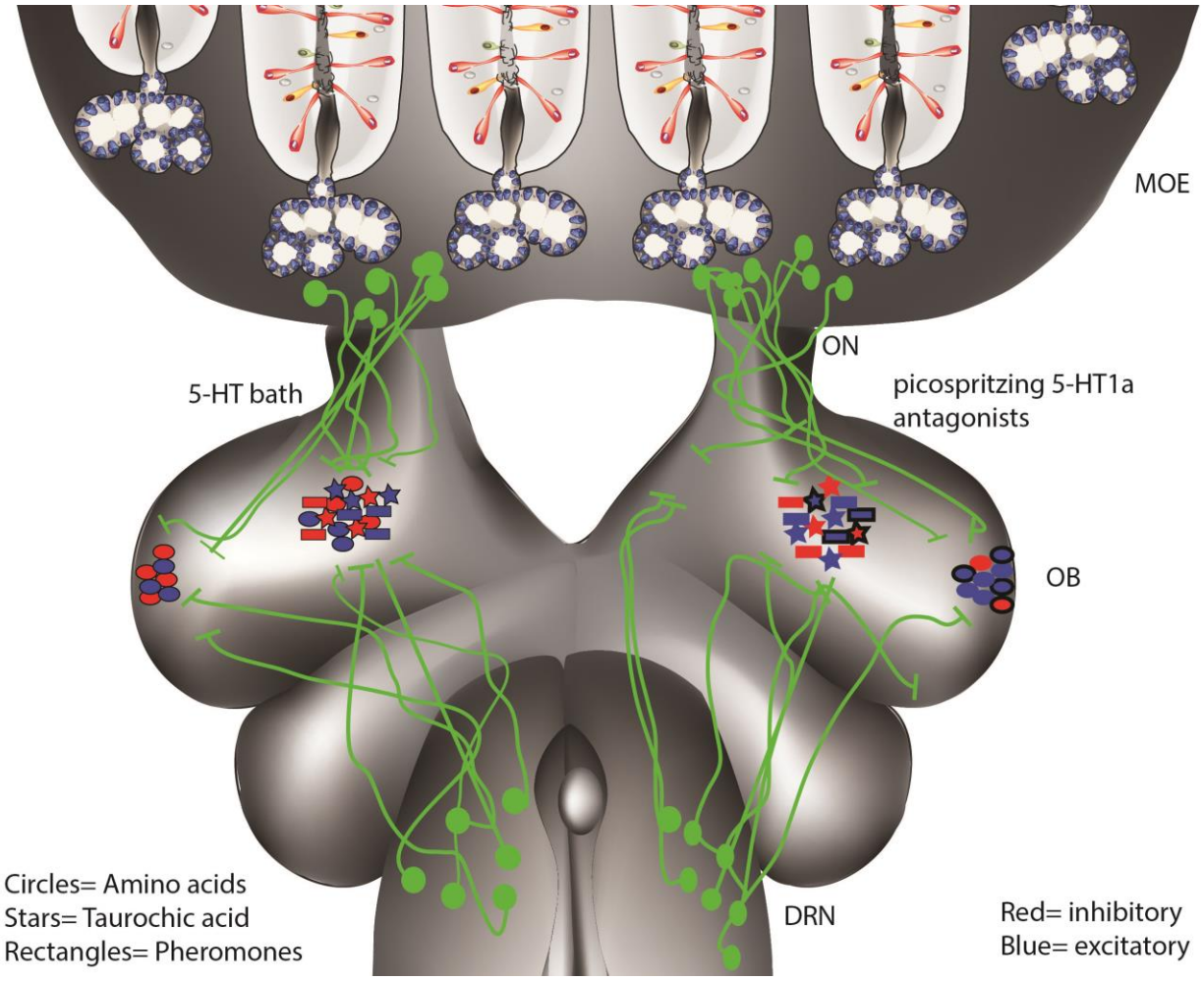


Figure 4.2. A summary diagram of the effect of 5-HT bath application and picospritzing of 5-HT1a antagonists on the temporal aspects of odour-evoked LFP responses in the olfactory bulb (OB). The 5-HT bath application (left OB) caused inhibitory effects (red) and excitatory effects (blue) on the number of peaks and response duration of amino acids (circles), pheromones (rectangles) and taurocholic acid (stars) in the dorsal OB and of amino acids in the lateral OB. Picospritzing (right bulb) of the 5-HT1a antagonist, s(-)-uh-301 (black outline), mostly caused excitatory effects on the temporal aspects of pheromones and taurocholic acid in the dorsal OB and amino acids in the lateral OB. However, picospritzing of the 5-HT1a antagonist, spiperone hydrochloride (no outline), caused excitatory and inhibitory effects on response duration and the number of peaks of taurocholic acid and pheromones in the dorsal OB and amino acids in the lateral OB. DRN- Dorsal Raphe Nuclei; MOE-main olfactory epithelium.

recordings were being taken. In experiment 2, the effect of picospritzing 5-HT1a antagonists predominantly caused excitatory effects on the peak amplitude. This signifies that the 5-HT1a antagonists acted on 5-HT1a receptors to reverse the inhibitory effect on peak amplitude caused by 5-HT meaning that it caused depolarization of the neurons being recorded from in the lamprey OB. These results are consistent with the mechanism of action of the 5-HT1a antagonist on the 5-HT1a receptor (described in section 1.6.1).

LFP recordings are useful for studying 5-HT modulation by bath application because they allow for an understanding of how modulatory circuits function to affect other neurons (Mazzoni et al., 2008). The 5-HT bath application could have been acting on a variety of different 5-HT sources in the OB that arise from different areas of the lamprey (see section 1.5.3). Since LFP's are capable of recording the synaptic activity from a multitude of neurons at once including excitatory and inhibitory interneurons that modulate the activity of other neurons (Mitzdorf 1985; Logothetis et al., 2008), they would be better equipped for determining the modulation caused by 5-HT on odour responses during a bath application. When using a technique such as patch-clamp or single unit recordings, the activity of the interneurons and their modulatory effects on other neurons would be difficult to identify (Buszaki 2006; Mazzoni et al., 2008). However, when examining 5-HT modulation at the level of the receptor, a technique such as patch-clamp or single unit recordings would be better suited. When investigating the effect of picospritzing the 5-HT1a antagonists on the lamprey ON, recordings should be taken from neurons that possess the 5-HT1a receptor. While the lamprey does have 5-HT1a receptors distributed throughout the OB (Barreiro-Iglesias et al., 2012), some recordings could have encountered activity from other 5-HT receptors in the OB since LFP's record from such a wide range of neurons, which could explain the inhibitory effects seen during antagonist application. In comparison, patch-clamp or

single unit recordings could measure the change in membrane potential of a single neuron that has the 5-HT1a receptor while that neuron is under modulation by the 5-HT1a antagonists. An additional setback of LFP recordings is that since they record from a multitude of neuronal processes in the lamprey OB, it can cause an obscure signal that is difficult to interpret and requires an in-depth analysis to understand the results. Since patch-clamp and single-unit recordings only record from a single neuron, the signal would be much clearer making the results easier to interpret.

While 5-HT caused a modulatory effect on peak amplitude in both experiment 1 and 2, the modulation was stronger and more consistent when the 5-HT1a blocker were picospritzed compared to the 5-HT bath application. In experiment 1, the benefit of applying 5-HT through bath application is that by submerging the brain-olfactory preparation in 5-HT, 5-HT fibers in the olfactory bulb will be stimulated since 5-HT is found throughout the lamprey OB (see section 1.5.3). However, a setback is that applying 5-HT through bath application will not localize the 5-HT modulatory effect to the lamprey OB. Di Prisco et al (1994) showed that some 5-HT cell bodies that originate in the brainstem have fibers that innervate onto reticulospinal cells in the spinal cord, which are responsible for movement. There are also dorsal column 5-HT fibers in the spinal cord that originate from dorsal root ganglion (Harris-Warrick et al., 1985; Zhang et al., 1996) and spinal 5-HT neurons that do not make any output synapses (Christenson et al., 1990; Schotland et al., 1996). Another setback of the 5-HT bath application is that it will not localize the modulatory effect to the particular 5-HT receptor of interest in the lamprey OB. There are 7 different 5-HT receptors, some of which have subreceptors, and some have been shown to cause excitatory effects, while others have displayed inhibitory effects (Hoyer et al., 1994; Barnes et al., 1999; Bockaert et al., 2006). While it is shown that the lamprey OB expresses the inhibitory 5-

HT1a receptor (Barreiro-Iglesias et al., 2012), the inhibitory and excitatory effects seen by the 5-HT bath application suggest the possibility of other 5-HT receptors being expressed in the OB. This scenario was observed in the previously described study on mouse brain slices by Stanford et al. (2005) where they found that the activation of different 5-HT receptors caused inhibitory and excitatory effects on the same subthalamic neurons at different times during 5-HT bath application. In addition, 5-HT levels could be higher during the adult spawning stage than the metamorphic transformer stage, which could pose as another explanation for the stronger modulatory effects seen during the picospritzing experiments.

In experiment 2, the benefit of picospritzing 5-HT1a antagonists into the ON is that it provides a more local application to examine 5-HT modulation on odour responses than offered by the 5-HT bath application. It eliminates the possibility that 5-HT modulation on odour responses could be caused by the 5-HT fibers that project into the OB from the dorsal raphe nuceli in the midbrain. However, a setback of this experiment could be picospritzing at the level of the ON instead of the OB itself. The ON of the sea lamprey does not contain any G-protein coupled 5-HT1a receptors since the axons running along the ON do not have the receptors. By picospritzing the antagonists directly into the OB, it ensures that the drug would be acting on the 5-HT1a receptors. Instead, by picospritzing into the ON, we relied on the drug migrating from the picospritzing location in the ON to the OB to act on the 5-HT1a receptors.

Both 5-HT1a antagonists had excitatory effects on the peak amplitude of odour-evoked responses from the OB. However, s(-)-uh-301 had stronger effects, causing excitatory effects on peak amplitude in every animal across all odours in both regions of the OB, while spiperone had a few recordings that showed inhibitory effects. A likely explanation for this is that s(-)-uh-301 acts specifically as a 5-HT1a receptor antagonist (Moreau et al., 1992), while spiperone has been

acts as a 5-HT1a receptor antagonist, a 5-HT2a receptor antagonist (Hoyer et al., 1994) and a dopamine 1 and dopamine 2 antagonist (Seeman et al., 1994). These findings suggest that when s(-)-uh-301 was picospritzed into the ON, it should have blocked the effect of 5-HT since it is a specific antagonist for the 5-HT1a receptor and the lamprey OB has the 5-HT1a receptor expression (Barreiro-Iglesias et al., 2012), which was confirmed by the results. Within the lamprey OB, the presence of dopaminergic neurons has been shown (Pierre et al., 1997). Therefore, when spiperone was applied to the ON, and diffused into to the OB, it could have acted on the dopamine 1 or 2 receptors instead of the 5-HT1a receptor. Additionally, spiperone acts as a 5-HT2a receptor antagonist and the 5-HT2a receptor has been shown to cause excitatory effects (Hoyer et al., 1994; Barnes et al., 1999; Bockaert et al., 2006). Since some recordings showed inhibitory effects on peak amplitude with the application of spiperone, this further supports the idea that other 5-HT receptors may be expressed in the lamprey OB. These differences between the antagonists may also explain why s(-)-uh-301 caused an excitatory effect on the response duration and number of peaks in a response for all odours in both regions of the OB, while spiperone only had an excitatory effect on amino acids in the lateral OB. Another explanation could be that 5-HT had stronger modulatory effects on amino acids than pheromones and taurocholic acid.

This is the first study that shows that 5-HT might modulate one odour class more effectively than other odours. In this study, a stronger modulatory effect on olfactory responses was seen on amino acids than pheromones and bile acids. Each receptor of a G-protein coupled olfactory sensory neuron that synapses onto mitral cells in glomeruli is capable of recognizing a wide range of different odour molecules (Floriano et al., 2000). It is possible that OSN's with receptors that have a high affinity for amino acids synapse onto mitral cells of glomeruli in the

olfactory bulb where the density of 5-HT fibers is highest. This means that olfactory sensory neurons that have a higher affinity for pheromones and bile acids could synapse into regions of the olfactory bulb that have a lower density of 5-HT fibers. On a behaviour level, L-amino acids have been shown to play a vital role in detecting food in the olfactory system of many species such as the blackspot sea brem (Hubbard et al., 2011), the catfish (Caprio 1978) and also the sea lamprey (Kleerekoper 1963). Therefore, it is possible that 5-HT acts to modulate lamprey behaviour when attempting to locate food. Perhaps something in the water in the vicinity of an unhealthy prey will stimulate 5-HT and cause it to deter the lamprey by inhibiting its ability to detect the amino acid released by the prey. 5-HT has also been shown to have a role in controlling satiety. It was shown that when 5-HT antagonists were applied to rats already in the state of satiety, there was an increase in food intake (Fletcher 1988). In humans, it was shown that 5-HT caused a decrease in feeding when satiety was reached (Blundell 1977). It is possible that 5-HT acts to modulate lamprey feeding behaviour once it reaches a state of satiety.

In previously described studies, 5-HT in the olfactory bulb of other vertebrate species showed that 5-HT was important for olfactory learning and remembering olfactory cues (McLean et al., 1993; Langdon et al., 1997; Ganesh et al., 2010). In the current study, it is shown that 5-HT modulates olfaction in the sea lamprey OB, so it is possible that 5-HT could play a crucial role in odour learning abilities and regulation of odour-evoked responses of the lamprey.

As previously discussed, researchers have discovered an increase in production of 5-HT in the brain during stressful periods in mammals and fish (see section 1.3). Specifically, the juvenile carp increases 5-HT production in its brain during periods of thermal stress (De Boeck et al., 1996). The lamprey is a temperature sensitive species (Meeuwig et al., 2005), so 5-HT

could play a role in modulating odour-induced behavioural responses in an environment that causes them thermal shock.

Polter et al. (2010) linked the 5-HT1a receptor to passive avoidance learning, which was previously described, in other species and Madjid et al. (2010) showed the receptor could have an effect on inhibitory learning. Based on these findings, 5-HT could inhibit odour responses in the OB of the lamprey as a way of avoiding harmful stimuli in the environment that the lamprey previously encountered.

We conclude that the data presented establishes that 5-HT does modulate olfactory responses in the OB of the sea lamprey via the 5-HT1a receptor and that 5-HT may also modulate olfactory-mediated behaviours. While it is shown that 5-HT displays a modulatory effect, the OB of the sea lamprey has a vast array of other modulatory networks such as GABA, dopamine and glutamate (Pierre et al., 1997; Meléndez-Ferro et al., 2001; Robertson et al., 2007; Dubuc et al., 2008). Future studies should investigate the modulatory effects caused by these neurotransmitters on odour responses using patch-clamp or single unit recordings in order to fully understand how modulatory circuits as a whole effect olfactory sensory processing in the sea lamprey olfactory bulb.

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Appendix

Table A1: The values for peak amplitude with their standard means and standard deviations for every odour in both regions of the olfactory bulb of all animals tested during the 5-HT bath application.

OB region	Odour	Animal #	Treatment	Peak Amplitude (µV) Del 1	Del 2	Del 3	Standard Mean	Standard Deviation
Dorsal	TCA	1	Control	-1.2784	-1.7042	-1.0156	-1.33273	0.283733
			5-HT bath	-1.0214	-0.6712	-0.7432	-0.81193	0.151004
			Washout	-1.2645	-0.5193	-0.8123	-0.86537	0.306532
		2	Control	-0.5005	-0.687	-0.4819	-0.55647	0.092613
			5-HT bath	-0.401	-0.2925	-0.3247	-0.3394	0.045498
			Washout	-0.3387	-0.3348	-0.3108	-0.3281	0.012336
		3	Control	-0.3678	-0.6428	-0.5307	-0.51377	0.112905
			5-HT bath	-0.9765	-0.5764	-0.9735	-0.84213	0.187906
			Washout	-0.7638	-0.4588	-0.7465	-0.65637	0.139879
		4	Control	-0.7157	-0.7935	-0.656	-0.72173	0.056296
			5-HT bath	-0.4416	-0.7626	-0.4969	-0.56703	0.140117
			Washout	-0.45	-0.2979	-0.625	-0.45763	0.133647
		5	Control	-0.7007	-0.8733	-0.6732	-0.74907	0.088561
			5-HT bath	-0.4939	-0.5476	-0.4332	-0.49157	0.046733
			Washout	-0.4483	-0.6844	-0.5432	-0.55863	0.097003
		6	Control	-0.5091	-0.4657	-0.4342	-0.46967	0.030706
			5-HT bath	-0.6042	-0.5253	-0.586	-0.57183	0.033733
			Washout	-0.4477	-0.4467	-0.3993	-0.43123	0.022584
		7	Control	-0.3355	-0.1912	-0.3139	-0.2802	0.063547
			5-HT bath	-0.2545	-0.2504	-0.3101	-0.27167	0.027228
			Washout	-0.1767	-0.3091	-0.2597	-0.2485	0.054629
		8	Control	-0.4065	-0.4619	-0.4561	-0.4415	0.024862

		5-HT bath	-0.3743	-0.4666	-0.4316	-0.42417	0.038046
		Washout	-0.3994	-0.4754	-0.4001	-0.42497	0.035663
	9	Control	-0.7125	-0.7875	-0.6788	-0.72627	0.045432
		5-HT bath	-1.0323	-0.7870	-0.6991	-0.83947	0.140997
		Washout	-0.976	-0.6225	-0.5785	-0.72567	0.177921
	10	Control	-0.3718	-1.2652	-0.475	-0.704	0.399059
		5-HT bath	-0.549	-1.5332	-0.5867	-0.88963	0.455331
		Washout	-0.3984	-0.9608	-0.4736	-0.61093	0.249291
	11	Control	-0.5432	-0.7111	-0.6542	-0.63617	0.069721
		5-HT bath	-0.4887	-0.6345	-0.3521	-0.49177	0.11531
		Washout	-0.5356	-0.5159	-0.4674	-0.5063	0.028658
	12	Control	-2.8029	-1.3468	-1.7313	-1.96033	0.616116
		5-HT bath	-0.7988	-2.396	-1.2585	-1.48443	0.67134
		Washout	-1.2717	-1.4213	-1.7154	-1.46947	0.184314
Pheromones	1	Control	-0.6962	-0.8312	-0.6064	-0.71127	0.092391
		5-HT bath	-0.5958	-0.3786	-0.3724	-0.44893	0.103881
		Washout	-0.3886	-0.3643	-0.3999	-0.38427	0.014853
	2	Control	-0.4649	-0.7633	-0.2940	-0.5074	0.193934
		5-HT bath	-0.4375	-0.9985	-0.5621	-0.66603	0.24053
		Washout	-0.922	-0.5091	-0.3452	-0.5921	0.242681
	3	Control	-0.4895	-0.798	-0.5432	-0.61023	0.134569
		5-HT bath	-0.4711	-0.5055	-0.3707	-0.4491	0.057188
		Washout	-0.4633	-0.4269	-0.5816	-0.4906	0.06604
	4	Control	-0.8524	-1.4763	-0.9761	-1.1016	0.269723
		5-HT bath	-0.5762	-1.345	-0.9288	-0.95	0.314219
		Washout	-0.8921	-1.5748	-0.9412	-1.13603	0.310902
	5	Control	-0.7274	-1.2012	-0.5663	-0.83163	0.269472

		5-HT bath	-1.9501	-0.9278	-1.2036	-1.3605	0.431847
		Washout	-1.0524	-1.5465	-0.4993	-1.03273	0.427744
	6	Control	-0.2044	-0.2173	-0.2323	-0.218	0.011401
		5-HT bath	-0.185	-0.1748	-0.1987	-0.18617	0.009792
		Washout	-0.317	-0.2769	-0.2388	-0.27757	0.031928
	7	Control	-0.2506	-0.2789	-0.3431	-0.29087	0.038699
		5-HT bath	-0.2833	-0.2000	-0.1923	-0.2252	0.041203
		Washout	-0.2596	-0.318	-0.2561	-0.2779	0.028391
	8	Control	-0.4816	-0.5459	-0.8255	-0.61767	0.149286
		5-HT bath	-0.5132	-0.6224	-0.5045	-0.5467	0.053646
		Washout	-0.4486	-0.5853	-0.5524	-0.52877	0.058256
	9	Control	-0.5318	-0.6523	-0.6172	-0.60043	0.050602
		5-HT bath	-0.5517	-0.6826	-0.6721	-0.63547	0.059387
		Washout	-0.7782	-0.5274	-0.4563	-0.5873	0.138072
	10	Control	-0.5839	-0.6076	-0.4353	-0.54227	0.076253
		5-HT bath	-0.4394	-0.3541	-0.4132	-0.40223	0.035677
		Washout	-0.5374	-0.4783	-0.4786	-0.4981	0.02779
	11	Control	-0.6558	-0.7503	-0.5153	-0.64047	0.096549
		5-HT bath	-0.845	-0.4627	-1.0578	-0.7885	0.246212
		Washout	-0.5675	-0.4286	-0.4270	-0.47437	0.065858
Amino Acids	1	Control	-0.9614	-0.3694	-0.6535	-0.66143	0.241748
		5-HT bath	-0.8943	-0.2456	-0.5040	-0.54797	0.266649
		Washout	-0.7345	-0.4544	-0.5893	-0.59273	0.114376
	2	Control	-0.5633	-0.4177	-0.3984	-0.4598	0.073608
		5-HT bath	-0.516	-0.3152	-0.3145	-0.3819	0.094823
		Washout	-0.2149	-0.3078	-0.3345	-0.28573	0.051259
	3	Control	-0.4986	-0.4829	-0.3826	-0.4547	0.051384

			5-HT bath	-0.3564	-0.4197	-0.3403	-0.37213	0.034271
			Washout	-0.2858	-0.482	-0.3789	-0.38223	0.080133
		4	Control	-0.5361	-0.9658	-0.3752	-0.6257	0.249297
			5-HT bath	-0.4837	-0.318	-0.3968	-0.3995	0.067674
			Washout	-0.2643	-0.3009	-0.2354	-0.26687	0.026802
Lateral	Amino Acids	1	Control	-0.4633	-0.9998	-0.8564	-0.77317	0.226795
			5-HT bath	-0.9494	-0.7078	-0.5692	-0.74213	0.157103
			Washout	-0.8564	-0.4932	-0.7354	-0.695	0.151003
		2	Control	-0.1989	-0.3356	-0.2785	-0.271	0.056059
			5-HT bath	-0.3246	-0.4178	-0.3431	-0.36183	0.040289
			Washout	-0.2863	-0.2421	-0.2167	-0.24837	0.028758
		3	Control	-0.9214	-0.8601	-0.7711	-0.85087	0.061706
			5-HT bath	-1.1228	-0.7257	-0.9458	-0.93143	0.162433
			Washout	-0.8875	-0.8616	-0.7284	-0.82583	0.069702
		4	Control	-0.6537	-1.1774	-0.9140	-0.91503	0.213801
			5-HT bath	-0.6931	-0.6533	-0.5953	-0.64723	0.040156
			Washout	-0.5400	-0.9729	-0.7553	-0.75607	0.176732
		5	Control	-0.7740	-1.0043	-1.1177	-0.96533	0.142995
			5-HT bath	-0.6208	-0.5557	-0.5100	-0.56217	0.045464
			Washout	-0.3892	-0.5930	-0.6940	-0.55873	0.126771
		6	Control	-0.8478	-1.0106	-0.7849	-0.8811	0.095103
			5-HT bath	-0.5322	-0.5099	-0.6116	-0.55123	0.043646
			Washout	-0.2897	-0.4057	-0.5950	-0.43013	0.12583
		7	Control	-0.7582	-0.7598	-0.8353	-0.78443	0.035974
			5-HT bath	-1.3551	-0.9455	-0.9442	-1.0816	0.193394
			Washout	-0.7870	-0.5678	-0.6695	-0.67477	0.089565
		8	Control	-2.109	-1.2180	-1.3884	-1.5718	0.386175

	5-HT bath	-1.1625	-1.0923	-1.0267	-1.09383	0.055451
	Washout	-0.9268	-0.622	-1.0462	-0.865	0.178607

Table A2: The values for the number of peaks with their standard means and standard deviations for every odour in both regions of the olfactory bulb of all animals tested during the 5-HT bath application.

OB region	Odour	Animal #	Treatment	# of Peaks Del 1	Del 2	Del 3	Standard Mean	Standard Deviation
Dorsal	TCA	1	Control	2	2	2	2	0
			5-HT bath	2	1	3	2	0.816497
			Washout	2	2	2	2	0
		2	Control	2	3	2	2.333333	0.471405
			5-HT bath	3	4	3	3.333333	0.471405
			Washout	2	3	3	2.666667	0.471405
		3	Control	9	5	4	6	2.160246
			5-HT bath	3	6	3	4	1.414213
			Washout	4	8	5	5.666667	1.699673
		4	Control	2	2	2	2	0
			5-HT bath	3	2	2	2.333333	0.471404
			Washout	2	3	3	2.666667	0.471404
		5	Control	3	1	2	2	0.816496
			5-HT bath	3	4	2	3	0.816496
			Washout	7	9	5	7	1.632993
		6	Control	2	2	2	2	0
			5-HT bath	3	3	2	2.666667	0.471404
			Washout	3	3	3	3	0
		7	Control	1	1	2	1.333333	0.471404
				1	1	1	1	0
			5-HT bath	2	2	1	1.666667	0.471404
		8	Washout	8	10	9	9	0.816496
			Control	8	8	7	7.666667	0.471404
			5-HT bath	8	14	11	11	2.449489
		9	Washout	8	8	9	8.333333	0.471404
			Control	6	6	10	7.333333	1.885618
			5-HT bath	4	7	6	5.666667	1.247219
		10	Washout	4	3	5	4	0.816496
			Control	4	5	3	4	0.816496
			5-HT bath	4	4	4	4	0

	11	Washout	10	9	8	9	0.816496
		Control	4	4	4	4	0
		5-HT bath	3	3	3	3	0
	12	Washout	1	2	1	1.333333	0.471404
		Control	3	1	1	1.666667	0.942809
		5-HT bath	2	1	1	1.333333	0.471404
Pheromones	1	Washout	3	3	3	3	0
		Control	2	2	2	2	0
		5-HT bath	1	3	2	2	0.816496
	2		2	1	4	2.333333	1.247219
		Washout	3	1	2	2	0.816496
		Control	1	2	1	1.333333	0.471404
	3	5-HT bath	3	3	3	3	0
		Washout	5	4	6	5	0.816496
		Control	5	6	4	5	0.816496
	4	5-HT bath	2	1	3	2	0.816496
		Washout	3	1	3	2.333333	0.942809
		Control	3	1	2	2	0.816496
	5	5-HT bath	5	4	6	5	0.816496
		Washout	1	3	3	2.333333	0.942809
		Control	3	1	2	2	0.816496
	6	5-HT bath	1	1	1	1	0
		Washout	1	3	2	2	0.816496
		Control	3	1	3	2.333333	0.942809
	7	5-HT bath	3	2	2	2.333333	0.471404
		Washout	1	2	1	1.333333	0.471404
		Control	1	1	1	1	0
	8	5-HT bath	4	7	3	4.666667	1.699673
		Washout	4	4	5	4.333333	0.471404
			3	3	3	3	0
	9	Control	8	7	7	7.333333	0.471404
		5-HT bath	6	3	4	4.333333	1.247219
		Washout	3	5	4	4	0.816496
	10	Control	4	4	4	4	0
		5-HT bath	7	4	5	5.333333	1.247219
		Washout	3	3	3	3	0
	11	Control	4	2	5	3.666667	1.247219
		5-HT bath	4	6	3	4.333333	1.247219
		Washout	6	4	3	4.333333	1.247219
Amino Acids	1	Control	1	2	1	1.333333	0.471405
		5-HT bath	1	1	1	1	0

			Washout	1	3	2	2	0.816496
		2	Control	3	3	3	3	0
			5-HT bath	1	2	2	1.666667	0.471404
			Washout	1	1	1	1	0
		3	Control	3	4	4	3.666667	0.471404
			5-HT bath	1	1	2	1.333333	0.471404
			Washout	1	1	1	1	0
		4	Control	2	4	5	3.666667	1.247219
			5-HT bath	2	6	6	4.666667	1.885618
			Washout	3	3	3	3	0
Lateral	Amino Acids	1	Control	5	2	2	3	1.414213
			5-HT bath	2	3	4	3	0.816496
			Washout	4	2	3	3	0.816496
		2	Control	1	1	2	1.333333	0.471404
			5-HT bath	3	1	2	2	0.816496
			Washout	5	3	4	4	0.816496
		3	Control	4	3	4	3.666667	0.471404
			5-HT bath	3	3	4	3.333333	0.471404
			Washout	4	4	5	4.333333	0.471404
		4	Control	7	5	6	6	0.816496
			5-HT bath	3	3	4	3.333333	0.471404
			Washout	1	1	1	1	0
		5	Control	4	3	5	4	0.816496
			5-HT bath	7	9	8	8	0.816496
			Washout	7	4	5	5.333333	1.247219
		6	Control	3	3	3	3	0
			5-HT bath	6	4	7	5.6666667	1.247219
			Washout	5	4	3	4	0.816496
		7	Control	5	4	3	4	0.816496
			5-HT bath	3	3	2	2.666667	0.471404
			Washout	5	4	4	4.333333	0.471404
		8	Control	1	4	3	2.666667	1.247219
			5-HT bath	2	2	2	2	0
			Washout	2	2	3	2.333333	0.471404

Table A3: The values for response duration with their standard means and standard deviations for every odour in both regions of the olfactory bulb of all animals tested during the 5-HT bath application.

OB region	Odour	Animal #	Treatment	Response Duration (ms) Del 1	Del 2	Del 3	Standard Mean	Standard Deviation
Dorsal	TCA	1	Control	247.5	196.5	222	222	20.82066
			5-HT bath	315.6	157.8	473.4	315.6	128.8432
			Washout	166.2	775.1	470.65	470.65	248.5824
		2	Control	827.7	1399.8	1113.75	1113.75	233.5588
			5-HT bath	1157.9	1069.4	1113.65	1113.65	36.12997
			Washout	2147.8	1262.6	819	1409.8	552.3756
		3	Control	2671.1	970.3	668.4	1436.6	881.5814
			5-HT bath	272.1	823.2	421.6	505.6333	232.7001
			Washout	922.5	1362.7	2511.6	1598.933	669.9076
		4	Control	391.2	195.6	586.8	391.2	159.7067
			5-HT bath	508	126	304.9	312.9667	156.0551
			Washout	238.1	1745.4	991.75	991.75	615.3526
		5	Control	2539.5	796.5	1668	1668	711.5768
			5-HT bath	2384.7	1192.35	3577.05	2384.7	973.5497
			Washout	2320.9	1160.45	3481.35	2320.9	947.5035
		6	Control	294.6	441.9	147.3	294.6	120.2699
			5-HT bath	1953.7	2195.8	1584	1911.167	251.5706
			Washout	834.4	2656.2	1745.3	1745.3	743.7467
		7	Control	88.3	35	158.1	93.8	50.40562
			5-HT bath	456.5	188.6	68.6	237.9	162.1511
			Washout	76.7	42.4	205.4	108.1667	70.16582
		8	Control	3862.1	4947.7	4404.9	4404.9	443.1943
			5-HT bath	5492.1	4428.3	2794.8	4238.4	1109.325
			Washout	3413.9	4087.6	3426.1	3642.533	314.7491

	9	Control	104.7	76.1	143.3	108.0333	27.53535
		5-HT bath	125.7	57.2	91.45	91.45	27.96501
		Washout	293.6	146.8	440.4	293.6	119.8617
	10	Control	1555.6	3409.5	3111.9	2803.917	823.6359
		5-HT bath	2156	2537.7	4052.8	2845.4	628.3069
		Washout	2371.5	3179.1	2775.3	4692.825	2110.789
	11	Control	5136.4	8084.3	6610.35	6610.35	1203.475
		5-HT bath	1855.7	2783.55	927.85	1855.7	757.5864
		Washout	2060	1241.2	1650.6	1650.6	334.2737
	12	Control	96.5	325.4	124.2	182.0333	102.0043
		5-HT bath	623.4	198.7	242.3	354.8	190.7611
		Washout	288.7	184.6	148.2	207.1667	59.53712
Pheromones	1	Control	773.5	935.8	877.9	862.4	67.15906
		5-HT bath	1136.6	274.9	203.7	538.4	423.9888
		Washout	79.6	1504.7	1314.4	966.2333	631.7397
	2	Control	1185.8	63.6	241.5	496.9667	492.4636
		5-HT bath	264.1	83.7	173.9	173.9	73.64799
		Washout	62.9	265.5	164.2	164.2	82.7111
	3	Control	91.4	131.7	1111.55	444.8833	471.6915
		5-HT bath	2042.1	1021.05	3063.15	2042.1	833.6838
		Washout	1069.4	534.7	1604.1	1069.4	436.5807
	4	Control	540	270	698.73	502.91	176.9823
		5-HT bath	1086.6	46	963.6	698.7333	464.2757
		Washout	1624.8	45.9	835.35	835.35	644.5832
	5	Control	2897.3	1311.7	981.3	1730.1	836.2846
		5-HT bath	37.1	1480.6	843.1	786.9333	590.6432
		Washout	520.5	1198.2	1983.2	1233.967	597.6801
	6	Control	100.2	127.3	113.75	113.75	11.06353

		5-HT bath	68.8	293.6	120.8	161.0667	96.08959
		Washout	111.6	99.5	603.4	271.5	234.7407
	7	Control	1303.2	888.3	1095.75	1095.75	169.3822
		5-HT bath	93.2	752.6	422.9	422.9	269.1989
		Washout	84.2	137.8	191.9	137.9667	43.9685
	8	Control	2621	3577	2507.4	2901.8	479.6857
		5-HT bath	2922.7	1536.4	2854.9	2438	638.1281
		Washout	2011.8	3567.3	905.6	2161.567	1091.783
	9	Control	4400.6	3685.2	4042.9	4042.9	292.0608
		5-HT bath	2800.5	2455.1	2627.8	2627.8	141.009
		Washout	1068.7	3075.5	2072.1	2072.1	819.2727
	10	Control	3224.1	2394.5	2809.3	2809.3	338.6828
		5-HT bath	3321.2	1705.2	2513.2	2513.2	659.7292
		Washout	1355.9	677.95	2033.85	1355.9	553.5439
	11	Control	996.8	250.3	1613.9	953.6667	557.5223
		5-HT bath	3917.4	1410.8	705.7	2011.3	1378.212
		Washout	1630.1	1101.1	687.1	1139.433	385.9312
Amino Acids	1	Control	79.6	196.6	313.6	196.6	95.5301
		5-HT bath	31.9	108.3	78.3	72.83333	31.42879
		Washout	57.3	267.8	478.3	267.8	171.8725
	2	Control	383.5	441.45	499.4	441.45	47.31598
		5-HT bath	94.1	588.8	341.45	341.45	201.9604
		Washout	88.55	84.6	92.5	88.55	3.225161
	3	Control	707.5	874.4	1997	1192.967	572.6058
		5-HT bath	33.1	43.9	293.4	123.4667	120.2419
		Washout	20	23.6	322.3	121.9667	141.6647
	4	Control	1929.1	4456.4	3012.5	3132.667	1035.259

			5-HT bath	955	2087.5	3115.9	2052.8	882.5249
			Washout	924	1627.1	1211.65	1254.25	288.6156
Lateral	Amino Acids	1	Control	1746.6	619.8	1109.3	1158.567	461.3314
			5-HT bath	2869.8	3381.5	3110.2	3120.5	209.0276
			Washout	2945	1764.1	2345.55	2351.55	482.1191
		2	Control	51.7	25.3	409.1	162.0333	175.0347
			5-HT bath	1451.8	40.7	714.5	735.6667	576.2736
			Washout	1551	1934.5	1818.2	1767.9	160.5524
		3	Control	1100.1	1078	1103.3	1093.8	11.24841
			5-HT bath	748.3	849.4	1361	986.2333	268.195
			Washout	1750.1	1641.1	1072.2	1487.8	297.2236
		4	Control	441.4	561.2	1344.2	782.2667	400.3455
			5-HT bath	2445.4	3473.5	2570.9	2829.933	457.9455
			Washout	1561.6	2287	1624.9	1824.5	328.0563
		5	Control	788.2	259.8	583.9	543.9667	217.5586
			5-HT bath	1094.7	2132.4	2024.2	1750.433	465.7728
			Washout	1550.6	1220.9	1707.6	1493.033	202.8212
		6	Control	524.2	837.4	1015.5	792.3667	203.0844
			5-HT bath	441.4	551.5	1428.8	807.2333	441.8064
			Washout	1903	1612.8	1569	1694.933	148.208
		7	Control	911.8	981.6	1428.8	1107.4	229.0436
			5-HT bath	2384.2	1334.2	1697.2	1805.2	435.4102
			Washout	933.5	1129.8	1025.53	1029.61	80.19105
		8	Control	90.3	1185.7	2488.8	1254.933	980.4065
			5-HT bath	223.8	225	224.8	224.5333	0.524934
			Washout	905.7	544	1819.5	1089.733	536.7347

Table A4: The values for peak amplitude with their standard means and standard deviations for every odour in both regions of the olfactory bulb of all animals tested during the picospritzing of the 5-HT1a antagonists, spiperone hydrochloride and s(-)-uh-301.

OB region	Odour	Antagonist	Animal #	Treatment	Peak Amplitude (µV) Del 1	Del 2	Del 3	Standard Mean	Standard Deviation
Dorsal	TCA	Spiperone	1	Control	-1.52	-1.47	-1.57	-1.52	0.040825
				Spiperone	-1.93	-1.46	-1.07	-1.48667	0.3516
				Washout	-0.76	-1.94	-2.24	-1.64667	0.638818
			2	Control	-1.81	-1.81	-0.96	-1.52667	0.400694
				Spiperone	-1.27	-1.01	-0.73	-1.00333	0.220504
				Washout	-1.02	-1.05	-0.62	-0.89667	0.196016
			3	Control	-1.02	-0.43	-0.8	-0.75	0.243447
				Spiperone	-0.91	-0.86	-1.07	-0.94667	0.089567
				Washout	-0.78	-0.49	-0.48	-0.58333	0.139124
			4	Control	-0.82	-0.79	-0.63	-0.74667	0.0834
				Spiperone	-1.85	-1.03	-1.73	-1.53667	0.361601
				Washout	-1.01	-1.03	-0.92	-0.98667	0.047842
			5	Control	-0.92	-1.93	-0.84	-1.23	0.496051
				Spiperone	-1.22	-1.3	-1.39	-1.30333	0.069442
				Washout	-0.93	-1.17	-1.25	-1.11667	0.135974
			6	Control	-1	-1.15	-0.6	-0.91667	0.23214
				Spiperone	-2.29	-1.15	-1.01	-1.48333	0.573256
				Washout	-0.64	-0.65	-0.6	-0.63	0.021602
			7	Control	-0.5	-0.54	-0.73	-0.59	0.100333
				Spiperone	-0.23	-0.37	-0.34	-0.31333	0.060185
				Washout	-0.35	-0.29	-0.46	-0.36667	0.070396
			8	Control	-0.46	-0.39	-0.35	-0.4	0.045461

			Spiperone	-0.52	-0.8	-0.47	-0.59667	0.14522
			Washout	-0.43	-0.63	-0.41	-0.49	0.099331
		9	Control	-0.55	-0.57	-0.46	-0.52667	0.047842
			Spiperone	-1.33	-0.96	-1.54	-1.27667	0.239768
			Washout	-0.7	-0.71	-0.94	-0.78333	0.110855
		10	Control	-0.84	-1.07	-0.83	-0.91333	0.110855
			Spiperone	-0.97	-1.1	-1.28	-1.11667	0.127105
			Washout	-0.98	-0.95	-1.38	-1.10333	0.196016
		11	Control	-0.71	-0.72	-0.79	-0.74	0.03559
			Spiperone	-0.86	-0.7	-0.52	-0.69333	0.138884
			Washout	-0.64	-0.48	-0.57	-0.56333	0.06549
	5-HT+ Spiperone	1	Control	-0.54	-0.47	-0.46	-0.49	0.03559
			5-HT + Spiperone	-0.5	-0.52	-0.49	-0.50333	0.012472
			Washout	-0.42	-0.43	-0.43	-0.42667	0.004714
		2	Control	-0.75	-0.81	-0.82	-0.79333	0.030912
			5-HT+ Spiperone	-0.89	-0.84	-0.67	-0.8	0.094163
			Washout	-0.67	-0.76	-0.98	-0.80333	0.130213
	DMSO	1	Control	-0.52	-0.36	-0.55	-0.47667	0.0834
			DMSO	-0.55	-0.42	-0.45	-0.47333	0.0556
			Washout	-0.43	-0.45	-0.37	-0.41667	0.0340
		2	Control	-0.82	-0.74	-0.91	-0.82333	0.069442
			DMSO	-0.64	-1.01	-0.83	-0.82667	0.15107
			Washout	-0.89	-0.76	-0.81	-0.82	0.053541
Pheromones	Spiperone	1	Control	-1.61	-0.78	-0.84	-1.07667	0.377918
			Spiperone	-1.56	-2.68	-1.65	-1.96333	0.50809
			Washout	-1.41	-0.7	-0.98	-1.03	0.292005
		2	Control	-0.92	-1.83	-1.05	-1.26667	0.401857
			Spiperone	-0.67	-2.84	-1.92	-1.81	0.889307
			Washout	-0.55	-0.59	-1.24	-0.79333	0.316263

			3	Control	-1.91	-1.08	-0.81	-1.26667	0.468069
				Spiperone	-0.98	-0.65	-0.97	-0.86667	0.153261
				Washout	-0.83	-0.78	-1.38	-0.99667	0.271825
			4	Control	-0.98	-3	-1.39	-1.79	0.871818
				Spiperone	-2.27	-3	-2.65	-2.64	0.298105
				Washout	-1.9	-1.08	-1.91	-1.63	0.38893
			5	Control	-0.5	-1.09	-0.71	-0.76667	0.244177
				Spiperone	-0.97	-0.8	-1.82	-1.19667	0.446194
				Washout	-1.04	-1.09	-0.85	-0.99333	0.103387
			6	Control	-0.62	-0.44	-0.64	-0.56667	0.089938
				Spiperone	-0.49	-2.12	-1.54	-1.38333	0.674603
				Washout	-0.75	-0.62	-0.74	-0.70333	0.059067
			7	Control	-0.49	-0.85	-0.78	-0.70667	0.155849
				Spiperone	-1.02	-1.2	-1.08	-1.1	0.074833
				Washout	-0.81	-0.59	-0.62	-0.67333	0.097411
		5-HT+ Spiperone	1	Control	-0.44	-0.46	-0.43	-0.44333	0.012472
				5-HT + Spiperone	-0.44	-0.45	-0.41	-0.43333	0.016997
				Washout	-0.41	-0.47	-0.55	-0.47667	0.057349
			2	Control	-1.23	-1.45	-1.33	-1.39	0.06
				5-HT+ Spiperone	-1.54	-1.29	-1.19	-1.34	0.147196
				Washout	-1.38	-1.49	-1.34	-1.40333	0.063421
		DMSO	1	Control	-1.17	-1.23	-0.98	-1.12667	0.106562
				Spiperone	-0.51	-0.90	-1.53	-0.98	0.420238
				Washout	-0.95	-0.91	-1.11	-0.99	0.08641
			2	Control	-1.14	-1.09	-0.98	-1.07	0.066833
				Spiperone	-0.86	-1.21	-1.17	-1.08	0.156418
				Washout	-0.93	-1.13	-1.24	-1.1	0.128323
Lateral	Amino Acids	Spiperone	1	Control	-1.71	-1.09	-1.27	-1.35667	0.260427
				Spiperone	-0.89	-0.95	-1.5	-1.11333	0.27451
				Washout	-1.17	-0.85	-0.98	-1	0.131403

			2	Control	-0.47	-0.49	-0.36	-0.44	0.057155
				Spiperone	-0.63	-0.7	-0.64	-0.65667	0.030912
				Washout	-0.5	-0.63	-0.62	-0.58333	0.059067
			3	Control	-1.56	-0.84	-0.63	-1.01	0.398246
				Spiperone	-0.94	-1.62	-0.76	-1.10667	0.370345
				Washout	-1.19	-1.53	-0.8	-1.17333	0.298254
			4	Control	-0.81	-0.72	-0.69	-0.74	0.05099
				Spiperone	-1.26	-0.98	-1.1	-1.11333	0.114698
				Washout	-0.94	-1.31	-1.29	-1.18	0.169902
			5	Control	-0.31	-0.24	-0.21	-0.25333	0.041899
				Spiperone	-0.3	-0.49	-0.53	-0.44	0.100333
				Washout	-0.85	-0.46	-0.44	-0.58333	0.188739
			6	Control	-0.53	-1.22	-1.51	-1.08667	0.411042
				Spiperone	-1.47	-0.81	-1.23	-1.17	0.272764
				Washout	-1.26	-1.15	-1	-1.13667	0.106562
		5-HT+ Spiperone	1	Control	-0.77	-0.8	-0.83	-0.8	0.024495
				5-HT + Spiperone	-0.74	-0.82	-0.89	-0.81667	0.061283
				Washout	-0.76	-0.74	-0.68	-0.72667	0.033993
			2	Control	-1.45	-1.76	-1.51	-1.57333	0.134247
				5HT+ Spiperone	-1.81	-1.38	-1.56	-1.58333	0.17632
				Washout	-1.54	-1.61	-1.58	-1.57667	0.028674
		DMSO	1	Control	-0.53	-1.22	-1.68	-1.14333	0.472605
				DMSO	-1.47	-0.81	-0.98	-1.08667	0.279802
				Washout	-1.26	-1.15	-1.00	-1.13667	0.106562
			2	Control	-1.86	-1.75	-1.90	-1.83667	0.063421
				DMSO	-1.82	-1.89	1.67	-1.79333	0.091773
				Washout	-1.97	1-.71	-1.85	-1.84333	0.106249
Dorsal	TCA	S(-)-UH-301	1	Control	-0.68	-0.68	-0.62	-0.66	0.028284
				S(-)-UH-301	-0.72	-0.68	-0.81	-0.73667	0.054365
				Washout	-0.6	-0.81	-0.73	-0.71333	0.086538

		2	Control	-0.5	-0.56	-0.49	-0.51667	0.030912
			S(-)-UH-301	-0.58	-0.64	-0.73	-0.65	0.061644
			Washout	-0.47	-0.46	-0.55	-0.49333	0.040277
		3	Control	-0.6	-0.55	-0.44	-0.53	0.066833
			S(-)-UH-301	-0.55	-0.92	-0.75	-0.74	0.151217
			Washout	-0.89	-0.69	-0.66	-0.74667	0.102089
		4	Control	-1.21	-1.48	-1.23	-1.30667	0.122837
			S(-)-UH-301	-1.01	-0.85	-1.74	-1.2	0.387384
			Washout	-1.47	-0.9	-1.57	-1.31333	0.295108
	S(-)-UH-301 into the OB	1	Control	-0.96	-0.89	-1.11	-0.986667	0.091773
			S(-)-UH-301	-2.13	-1.87	-2.89	-2.296667	0.432769
			Washout	-1.23	-1.08	-0.67	-0.993333	0.23669
	Ethanol	1	Control	-1.41	-1.62	-1.37	-1.46667	0.109646
			S(-)-UH-301	-1.11	-1.14	-1.74	-1.33	0.290172
			Washout	-1.44	-1.48	-1.67	-1.53	0.100333
		2	Control	-0.67	-0.76	-0.71	-0.71333	0.036818
			S(-)-UH-301	-0.58	-0.95	-0.87	-0.8	0.158955
			Washout	-0.78	-0.69	-0.85	-0.77333	0.06549
Pheromones	S(-)-UH-301	1	Control	-0.84	-0.71	-0.66	-0.73667	0.075865
			S(-)-UH-301	-0.72	-0.82	-0.71	-0.75	0.049666
			Washout	-0.5	-0.51	-0.85	-0.62	0.162686
		2	Control	-0.6	-0.82	-0.68	-0.7	0.090921
			S(-)-UH-301	-0.84	-0.96	-0.74	-0.84667	0.089938
			Washout	-0.72	-0.69	-0.84	-0.75	0.064807
		3	Control	-0.47	-0.49	-0.44	-0.46667	0.020548
			S(-)-UH-301	-0.74	-0.43	-0.48	-0.55	0.135892
			Washout	-0.99	-0.35	-0.55	-0.63	0.267333

		Ethanol	1	Control	-0.67	-1.85	-1.34	-1.28667	0.483207
				S(-)-UH-301	-1.15	-1.32	-1.43	-1.3	0.115181
				Washout	-1.18	-0.92	-1.56	-1.22	0.262805
			2	Control	-1.56	-1.32	1-.44	-1.44	0.09798
				S(-)-UH-301	-1.67	-1.34	-1.51	-1.50667	0.134743
				Washout	-1.45	-1.67	-1.75	-1.62333	0.126842
Lateral	Amino Acids	S(-)-UH-301	1	Control	-0.79	-0.66	-0.62	-0.69	0.072572
				S(-)-UH-301	-0.96	-0.93	-1.04	-0.97667	0.046428
				Washout	-0.68	-0.98	-0.69	-0.78333	0.139124
			2	Control	-0.87	-1.05	-1.06	-0.99333	0.087305
				S(-)-UH-301	-1.15	-1.59	-1.89	-1.54333	0.303901
				Washout	-1.27	-1.57	-1.17	-1.33667	0.169967
			3	Control	-0.84	-1.08	-0.89	-0.93667	0.103387
				S(-)-UH-301	-1.07	-0.84	-1.22	-1.04333	0.156276
				Washout	-1.13	-0.97	-0.58	-0.89333	0.230988
			4	Control	-0.71	-0.82	-0.91	-0.81333	0.081786
				S(-)-UH-301	-0.85	-0.94	-1.33	-1.04	0.208327
				Washout	-1.07	-1.13	-0.46	-0.88667	0.302692
		Ethanol	1	Control	-1.43	-1.47	-1.56	-1.48667	0.054365
				S(-)-UH-301	-1.34	-1.57	-1.54	-1.48333	0.102089
				Washout	-1.75	-1.31	-1.52	-1.52667	0.179691
			2	Control	-0.56	-0.65	-0.62	-0.61	0.037417
				S(-)-UH-301	-0.46	-0.76	-0.53	-0.58333	0.128149
				Washout	-0.45	-0.67	-0.69	-0.60333	0.10873

Table A5: The values for the number of peaks with their standard means and standard deviations for every odour in both regions of the olfactory bulb of all animals tested during the picospritzing of the 5-HT1a antagonists, spiperone hydrochloride and s(-)-uh-301.

OB region	Odour	Antagonist	Animal #	Treatment	# of peaks			Standard Mean	Standard Deviation
					Del 1	Del 2	Del 3		
Dorsal	TCA	Spiperone	1	Control	3	3	3	3	0
				Spiperone	1	2	3	2	0.816497
				Washout	1	2	2	1.666667	0.471405
			2	Control	1	2	4	2.333333	1.247219
				Spiperone	3	5	5	4.333333	0.942809
				Washout	2	2	4	2.666667	0.942809
			3	Control	1	2	3	2	0.816497
				Spiperone	2	4	4	3.333333	0.942809
				Washout	2	2	3	2.333333	0.471405
			4	Control	5	7	8	6.666667	1.247219
				Spiperone	2	4	4	3.333333	0.942809
				Washout	6	7	8	7	0.816497
			5	Control	3	2	4	3	0.816497
				Spiperone	4	4	4	4	0
				Washout	3	2	3	2.666667	0.471405
			6	Control	1	1	1	1	0
				Spiperone	1	1	2	1.333333	0.471405
				Washout	2	2	3	2.333333	0.471405
			7	Control	11	8	7	8.666667	1.699673
				Spiperone	4	5	7	5.333333	1.247219
				Washout	5	4	6	5	0.816497
			8	Control	2	1	2	1.666667	0.471405

			Spiperone	4	1	4	3	1.414214
			Washout	3	1	2	2	0.816497
		9	Control	3	6	5	4.666667	1.247219
			Spiperone	1	3	5	3	1.632993
			Washout	4	3	3	3.333333	0.471405
		10	Control	3	3	3	3	0
			Spiperone	4	3	3	3.333333	0.471405
			Washout	3	3	2	2.666667	0.471405
		11	Control	4	3	3	3.333333	0.471405
			Spiperone	3	4	6	4.333333	1.247219
			Washout	5	4	6	5	0.816497
	5-HT+ Spiperone	1	Control	13	13	11	12.33333	0.942809
			5-HT + Spiperone	15	9	12	12	2.44949
			Washout	13	9	7	9.666667	2.494438
		2	Control	6	3	4	4.333333	1.247219
			5-HT + Spiperone	3	4	7	4.666667	1.699673
			Washout	5	5	6	5.333333	0.471405
	DMSO	1	Control	6	2	2	3.333333	1.885618
			DMSO	5	3	4	4	0.816497
			Washout	5	2	2	3	1.414214
		2	Control	4	5	4	4.333333	0.4714045
			DMSO	3	6	5	4.666667	1.2472191
			Washout	5	5	7	5.666667	0.942809
Pheromones	Spiperone	1	Control	1	1	1	1	0
			Spiperone	1	1	2	1.333333	0.471405
			Washout	1	1	1	1	0
		2	Control	1	1	2	1.333333	0.471405
			Spiperone	2	2	4	2.666667	0.942809
			Washout	2	3	4	3	0.816497
		3	Control	1	3	3	2.333333	0.942809

				Spiperone	2	3	3	2.666667	0.471405
				Washout	2	2	3	2.333333	0.471405
			4	Control	2	3	6	3.666667	1.699673
				Spiperone	2	2	3	2.333333	0.471405
				Washout	3	3	5	3.666667	0.942809
			5	Control	2	3	2	2.333333	0.471405
				Spiperone	2	3	3	2.666667	0.471405
				Washout	3	3	4	3.333333	0.471405
			6	Control	2	2	2	2	0
				Spiperone	1	1	3	1.666667	0.942809
				Washout	2	2	2	2	0
			7	Control	2	2	5	3	1.414214
				Spiperone	2	2	3	2.333333	0.471405
				Washout	2	3	3	2.666667	0.471405
		5-HT+ Spiperone	1	Control	11	10	11	10.66667	0.471405
				5-HT + Spiperone	17	9	18	14.66667	4.027682
				Washout	9	7	10	8.666667	1.247219
			2	Control	6	9	8	7.666667	1.247219
				5-HT + Spiperone	4	8	10	7.333333	2.494438
				Washout	7	7	7	7	0
		DMSO	1	Control	2	4	4	3.333333	0
				5-HT + Spiperone	3	4	3	3.333333	0
				Washout	3	4	3	3.333333	0
			2	Control	7	4	5	5.333333	1.2472191
				5-HT + Spiperone	3	6	7	5.333333	1.6996732
				Washout	6	6	6	6	0
Lateral	Amino Acids	Spiperone	1	Control	3	11	13	9	4.320494
				Spiperone	8	10	9	9	0.816497
				Washout	6	9	9	8	1.414214
			2	Control	2	2	5	3	1.414214

				Spiperone	3	5	6	4.666667	1.247219
				Washout	4	4	5	4.333333	0.471405
			3	Control	2	3	4	3	0.816497
				Spiperone	2	4	4	3.333333	0.942809
				Washout	2	2	5	3	1.414214
			4	Control	6	6	6	6	0
				Spiperone	8	5	7	6.666667	1.247219
				Washout	4	5	6	5	0.816497
			5	Control	2	2	2	2	0
				Spiperone	2	3	3	2.666667	0.471405
				Washout	2	3	3	2.666667	0.471405
			6	Control	2	2	2	2	0
				Spiperone	4	4	7	5	1.414214
				Washout	4	4	6	4.666667	0.942809
		5-HT+ Spiperone	1	Control	9	9	8	8.666667	0.471405
				5-HT + Spiperone	7	8	6	7	0.816497
				Washout	10	10	8	9.333333	0.942809
			2	Control	9	9	8	8.6666667	0.47140452
				5-HT+ Spiperone	7	9	10	8.6666667	1.24721913
				Washout	5	11	8	8	2.44948974
		DMSO	1	Control	2	2	3	2.3333333	0
				5-HT+ Spiperone	2	2	3	2.3333333	0
				Washout	2	2	3	2.3333333	0
			2	Control	5	7	9	7	0
				5-HT+ Spiperone	7	7	7	7	0
				Washout	6	5	9	6.666667	1.69967
Dorsal	TCA	S(-)-UH-301	1	Control	4	6	7	5.666667	1.247219
				S(-)-UH-301	7	7	8	7.333333	0.471405
				Washout	7	8	8	7.666667	0.471405

		2	Control	2	4	5	3.666667	1.247219
			S(-)-UH-301	3	4	5	4	0.816497
			Washout	2	3	5	3.333333	1.247219
		3	Control	2	3	4	3	0.816497
			S(-)-UH-301	2	4	4	3.333333	0.942809
			Washout	1	2	2	1.666667	0.471405
		4	Control	6	9	10	8.333333	1.699673
			S(-)-UH-301	4	7	8	6.333333	1.699673
			Washout	2	8	5	5	2.44949
	S(-)-UH-301 into the OB	1	Control	4	3	5	4	0.8164966
			S(-)-UH-301	3	4	4	3.666667	0.4714045
			Washout	5	2	4	3.666667	1.2472191
	Ethanol	1	Control	4	3	7	4.666667	1.6996732
			Ethanol	5	4	6	5	0.8164966
			Washout	7	3	5	5	1.6329932
		2	Control	4	4	6	4.666667	0.942809
			Ethanol	5	4	3	4	0.8164966
			Washout	7	4	5	5.333333	1.2472191
Pheromones	S(-)-UH-301	1	Control	4	6	7	5.666667	1.247219
			S(-)-UH-301	6	7	8	7	0.816497
			Washout	7	8	8	7.666667	0.471405
		2	Control	3	3	5	3.666667	0.942809
			S(-)-UH-301	3	5	5	4.333333	0.942809
			Washout	3	3	4	3.333333	0.471405
		3	Control	1	1	2	1.333333	0.471405
			S(-)-UH-301	2	2	3	2.333333	0.471405
			Washout	1	1	2	1.333333	0.471405
	Ethanol	1	Control	3	4	5	4	0.816497
			Ethanol	1	3	3	2.333333	0.942809
			Washout	4	3	2	3	0.816497

			2	Control	7	6	6	6.333333	0.471405
				Ethanol	5	9	8	7.333333	1.699673
				Washout	6	6	8	6.666667	0.942809
Lateral	Amino Acids	S(-)-UH-301	1	Control	3	6	6	5	1.414214
				S(-)-UH-301	3	4	4	3.666667	0.471405
				Washout	3	4	4	3.666667	0.471405
			2	Control	2	2	4	2.666667	0.942809
				S(-)-UH-301	3	4	5	4	0.816497
				Washout	2	3	3	2.666667	0.471405
			3	Control	2	2	6	3.333333	1.885618
				S(-)-UH-301	2	4	6	4	1.632993
				Washout	1	4	4	3	1.414214
			4	Control	7	8	9	8	0.816497
				S(-)-UH-301	7	10	11	9.333333	1.699673
				Washout	1	4	6	3.666667	2.054805
		Ethanol	1	Control	4	3	4	3.666667	0.471405
				Ethanol	2	5	4	3.666667	1.247219
				Washout	4	4	6	4.666667	0.942809
			2	Control	7	8	10	8.333333	1.247219
				Ethanol	9	7	9	8.333333	0.942809
				Washout	7	8	9	8	0.816497

Table A6: The values for the response duration with their standard means and standard deviations for every odour in both regions of the olfactory bulb of all animals tested during the picospritzing of the 5-HT1a antagonists, spiperone hydrochloride and s(-)-uh-301.

OB region	Odour	Antagonist	Animal #	Treatment	Response Duration (ms)			Standard Mean	Standard Deviation
					Del 1	Del 2	Del 3		
Dorsal	TCA	Spiperone	1	Control	738.9	847.9	1055.9	880.9	131.5016
				Spiperone	104.5	384.8	884.5	457.9333	322.6054

	Washout	286 361.5 636.6	428.0333	150.6654
2	Control	161 808.7 1180.2	716.6333	421.1487
	Spiperone	1267.1 1351.8 2736.9	1785.267	673.7942
	Washout	707.7 1754.8 1944.8	1469.1	543.95
3	Control	148.5 977 546.3	557.2667	338.3226
	Spiperone	354 857.6 712.1	641.2333	211.6125
	Washout	518.9 730.5 612.1	620.5	86.5893
4	Control	2039.1 1057.3 1205.1	1433.833	432.2206
	Spiperone	1171.2 149.1 775.3	698.5333	420.7865
	Washout	2219.6 1650.9 1528.2	1799.567	301.2029
5	Control	1145.7 534.9 634	771.5333	267.6512
	Spiperone	1161.5 689.9 959.9	937.1	193.2037
	Washout	1280.5 966.1 1112.5	1119.7	128.4542
6	Control	216.1 551.5 194.9	320.8333	163.3354
	Spiperone	167.1 2309.4 85.9	854.1333	1029.563
	Washout	978.9 2171.6 601.4	1250.633	669.2091
7	Control	3171.5 1670.3 2004	2281.933	643.6021
	Spiperone	2315.2 519.6 784	1206.267	791.5288
	Washout	2977.6 543.2 1171.2	1564	1031.922
8	Control	189.1 552.2 2897.4	1212.9	1200.31
	Spiperone	113 1547.2 1969.3	1209.833	794.4913
	Washout	274.4 639.1 761.5	558.3333	206.8962
9	Control	1616.2 2063 882.2	1520.467	486.7894
	Spiperone	1367.1 1642.7 135	1048.267	655.5053
	Washout	948 1377.3 933.1	1086.133	205.9758
10	Control	1706.6 1045.9 259.7	1004.067	591.4347

			Spiperone	932.1 837.7 923.9	897.9	42.69926
			Washout	1091.8 945.1 754.7	930.5333	138.0054
		11	Control	748 1669.1 2537.9	1651.667	730.8276
			Spiperone	1461.9 1473.9 2878.5	1938.1	664.9813
			Washout	1744.6 1998 2145.9	1962.833	165.7065
	5-HT+ Spiperone	1	Control	5292.7 5365.4 5596.3	5418.133	129.4317
			5-HT + Spiperone	4218.6 6364.6 7332.8	5972	1301.323
			Washout	5705.4 6225.9 6795.9	6242.4	445.3476
		2	Control	6553.1 5542.9 5432.1	5842.7	504.3612
			5-HT + Spiperone	5762.1 7541.2 4763.2	6022.1667	1148.926
			Washout	4897.3 7432.1 6333.2	6220.8667	1037.872
	DMSO	1	Control	347.2 1833 2484.7	1554.9667	894.50301
			DMSO	930.2 1729.4 2102.1	1507.275	488.87348
			Washout	588.9 2115.3 2816.8	1840.333	930.08588
		2	Control	3219.2 2452.2 2831.9	2834.433	313.13156
			DMSO	2190.2 3723.1 2912.9	2942.067	626.14355
			Washout	1987.2 4532.1 3121.2	3213.5	1040.999
Pheromones	Spiperone	1	Control	154.5 155.2 555.7	288.4667	188.9627
			Spiperone	87.9 137.5 54	93.13333	34.289
			Washout	141.3 166.3 268	191.8667	54.79333
		2	Control	125.4 1484.8 116	575.4	643.0544
			Spiperone	111.1 1253.2 513.8	626.0333	472.9661
			Washout	537.8 3442.8 856.1	1612.233	1300.912
		3	Control	143.7 633.5 2523.4	1100.2	1026.028
			Spiperone	353.6 401.7 891.8	549.0333	243.1668
			Washout	387 596.3 749.8	577.7	148.6953
		4	Control	144.2 374.3 1021.1	513.2	371.2217
			Spiperone	135.8 222.5 2229.6	862.6333	967.2392
			Washout	315.6 539.6 1087.3	647.5	324.1523

			5	Control	1106.2 994.1 937.5	1012.6	70.10283
				Spiperone	1164.4 894.7 685	914.7	196.2245
				Washout	1321.3 986.5 470.6	926.1333	349.9102
			6	Control	324.6 446 1234.4	668.3333	403.3262
				Spiperone	169.8 227.5 1753.6	716.9667	733.3889
				Washout	1963.3 2473.9 2550.7	2329.3	260.6934
			7	Control	427 251.8 1345.2	674.6667	479.5032
				Spiperone	545.4 293 1461.1	766.5	501.8488
				Washout	774.8 369.2 1419.2	854.4	432.3402
		5-HT+ Spiperone	1	Control	4722.9 3930.8 3907.2	4186.967	379.0845
				5-HT + Spiperone	7890.8 6251.2 1984.9	5375.633	2489.294
				Washout	3225.6 2770.6 2415.8	2804	331.442
			2	Control	3453.2 4124.3 3902.1	3826.5333	279.1374
				5-HT + Spiperone	2435.2 3432.9 4832.1	3566.7333	983.0958
				Washout	3322.1 3569.8 5342.1	4078	899.5556
		DMSO	1	Control	478.8 850.7 634.2	654.56667	152.509
				DMSO	648.4 796.8 614.3	686.5	79.22655
				Washout	714.6 767.7 643.2	708.5	51.00961
			2	Control	5552.1 2490.5 4431.1	4157.9	1264.7338
				DMSO	3898.2 4863.1 3421.8	4061.033	599.56787
				Washout	3332.1 3759.3 4932.1	4007.833	676.42522
Lateral	Amino Acids	Spiperone	1	Control	425.2 2547 4441	2471.067	1640.322
				Spiperone	2107 2820 2987.9	2638.3	381.8877
				Washout	1803 2399.8 3024.2	2409	498.5953
			2	Control	547.5 406.5 908.6	620.8667	211.4444
				Spiperone	2025 1285.8 2372	1894.267	452.9724
				Washout	2643.3 1623.4 3326.5	2531.067	699.8022
			3	Control	174.3 332.4 1860.6	789.1	760.4091

				Spiperone	677 2969.8 1256.9	1634.567	973.3814
				Washout	335.9 568.4 2633.7	1179.333	1032.764
			4	Control	712.3 1438.5 566.2	905.6667	381.4619
				Spiperone	562.8 1097.9 555.3	738.6667	254.0348
				Washout	379.3 1504.5 560.9	814.9	493.2246
			5	Control	1682.6 1569.4 1448.4	1566.8	95.62942
				Spiperone	2160.6 1456.7 1285.5	1634.267	378.6797
				Washout	1361.2 1302.1 2160.4	1607.9	391.4208
			6	Control	493.3 709.7 516	573	97.10472
				Spiperone	2026.3 2363 557.3	1648.867	783.9983
				Washout	1011.5 2509.5 1347.1	1622.7	641.8554
		5-HT+ Spiperone	1	Control	2596.8 3644.9 1597.8	2613.167	835.8052
				5-HT + Spiperone	2459.8 2589.1 1675.2	2241.367	403.8054
				Washout	2152.7 3162.3 1895.8	2403.6	546.6374
			2	Control	6352.9 4777.9 4892	5340.933	717.083
				5-HT + Spiperone	5490.1 5321.3 5873.2	5561.533	230.9047
				Washout	4988.7 5774.3 5931.2	5564.733	412.3229
		DMSO	1	Control	566.2 595 712.3	624.5	63.1875
				DMSO	383.6 555.3 1097.9	678.9333	304.4339
				Washout	379.3 560.9 1504.5	814.9	493.2246
			2	Control	1117.2 2453.2 1999.7	1856.7	554.71359
				DMSO	2431.2 1349.5 2198.3	1993	464.85108
				Washout	2234.1 1982.6 2837.2	2351.3	358.5965
Dorsal	TCA	S(-)-UH-301	1	Control	1311.6 2278.8 2270.7	1953.7	454.0453
				S(-)-UH-301	1729.8 2091 1953.9	1924.9	148.8783
				Washout	2507.1 1033.2 1702.8	1747.7	602.5542
			2	Control	609 905.5 2121	1211.833	654.1743
				S(-)-UH-	1376.7 1816.8 1980	1724.5	254.7969

			301 Washout	889 462.1 2097.4	1149.5	692.5541
		3	Control	1386.2 982.5 529.6	966.1	349.8977
			S(-)-UH-301	1628.1 1188.6 543.4	1120.033	445.4732
			Washout	773.4 666.6 143.1	527.7	275.4263
		4	Control	1950.3 2796 3821.9	2856.067	765.2571
			S(-)-UH-301	2293.4 3571.3 4971.6	3612.1	1093.751
			Washout	6206 495.4 1023.1	2574.833	2576.645
	S(-)-UH-301 into the OB	1	Control	3245.3 4532.1 3745.9	3841.1	529.6293
			S(-)-UH-301	4432.1 4909.3 3348.6	4230	652.9826
			Washout	4324.1 3329.9 4123.1	3925.7	429.2113
	Ethanol	1	Control	568.8 665.1 848.6	694.1667	116.0622
			Ethanol	651.3 652 792.2	698.5	66.25652
			Washout	601.2 793.9 693.6	696.2333	78.69148
		2	Control	992.8 1029.3 1239.2	1087.1	108.5783
			Ethanol	1029.4 1832.1 646.7	1169.4	493.959
			Washout	1116.7 1432.6 1398.2	1315.833	141.5071
Pheromones	S(-)-UH-301	1	Control	1328.1 327 2690.4	1448.5	968.6028
			S(-)-UH-301	1846.3 1269.2 2053.7	1723.067	331.9135
			Washout	1845 1396.2 2779.9	2007.033	576.3954
		2	Control	1056.6 1415.2 2299.9	1590.567	522.5028
			S(-)-UH-301	1014.3 1442.7 1728.2	1395.067	293.3883
			Washout	425.4 1041.1 1266.9	911.1333	355.6206
		3	Control	212.4 146.2 409.2	255.9333	111.6949
			S(-)-UH-301	100 111.1 383.9	198.3333	131.2937
			Washout	457.6 162.4 854	491.3333	283.3503
	Ethanol	1	Control	564.8 756.2 666.9	662.6333	78.19695
			Ethanol	643.3 732.9 645.7	673.9667	41.68368

				Washout	458.9 873.2 543.1	649.5167	132.0981
			2	Control	1119.4 1649.8 1435.4	1401.533	217.85508
				Ethanol	1029.5 1329.1 1639.9	1332.833	249.20874
				Washout	879.2 1776.4 1564.4	1406.667	382.88537
Lateral	Amino Acids	S(-)-UH-301	1	Control	1974.7 1711 663.4	1449.7	566.3245
				S(-)-UH-301	1553.6 1504 679.2	1245.6	401.0168
				Washout	1564 1323.5 1298.8	1395.433	119.6204
			2	Control	527.3 872.5 1305.2	901.6667	318.2453
				S(-)-UH-301	954.7 1563.3 1791.2	1436.4	353.0918
				Washout	909.8 580.3 547.6	679.2333	163.5809
			3	Control	735.3 113.8 4877.9	1909	2114.606
				S(-)-UH-301	582.9 2815 3338.5	2245.467	1194.88
				Washout	1573.4 136 2210.5	1306.633	867.6639
			4	Control	1916.1 1970.9 2364.4	2083.8	199.6714
				S(-)-UH-301	2467.3 2999.1 3709.7	3058.7	508.9555
				Washout	215.2 1789.6 2199.8	1401.533	855.4165
		Ethanol	1	Control	5673.1 5321.9 5893.5	5629.5	235.3825
				Ethanol	6372.9 4673.3 5632.1	5559.433	695.7588
				Washout	5198.3 5983.2 6531.3	5904.267	547.0497
			2	Control	3213.3 3892.1 3564.9	3556.767	277.17861
				Ethanol	2985.7 3282.8 3632.4	3300.3	264.304
				Washout	4321.4 2672.7 3587.3	3527.133	674.42219

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